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INTERSPECIFIC HYBRIDIZATION IN GOSSYPIUM AND THE MEIOTIC BEHAVIOR OF F_1 PLANTS[1]

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INTRODUCTION

The present study deals with F_1 hybrids between and within the following five morphologically distinct groups of *Gossypium:* (1) Cultivated American species; (2) wild American species; (3) cultivated Asiatic species; (4) a wild Australian species, *G. sturtii* F. Muell.; and (5) a wild American plant, *Thurberia thespesioides* A. Gray, possibly congeneric with *Gossypium.* Each of these five groups, with one exception, has the haploid chromosome number 13; in the cultivated American group, the haploid chromosome number is 26. The scientific and economic importance of the groups is indicated by the rapidly increasing amount of literature dealing with them.

Previous cytological studies of *Gossypium* have shown the relationships and possible origin of the species in several groups. Skovsted (*23*)[2] points out that Lawrence (*18*) suggests the possibility that Asiatic cottons are secondary polyploids. Lawrence's conclusions are based upon the chromosome association depicted by Denham (*18, p. 367*) and the complex inbreeding results obtained by Harland (*18, p. 376*). The occurrence of trisomes in a haploid cultivated American cotton, together with genetical data, led Skovsted to support Lawrence's assumption. Skovsted offers two alternative explanations of the origin of certain Asiatic species: (1) The doubling of chromosomes following the "crossing of two different but closely allied 7-chromosome species with the same 6-chromosome species" and (2) the doubling of a complement "composed of two similar sets of 6, and one chromosome appearing a third time."

During meiosis interspecific Asiatic hybrids have generally been reported to form 13 pairs of chromosomes and to exhibit normal cytological behavior (*1, 4*). Skovsted (*23*), however, records a decrease in the number of chiasmata in each pair of chromosomes and a very slight irregularity in first anaphase distribution. In a triploid Asiatic hybrid Skovsted found that polysomes up to septivalents were formed. Skovsted's findings demonstrate that autosyndesis occurred between the chromosomes in a haploid set, and further support the assumption that Asiatic species are of polyploid nature.

Davie (*3*) suggests that the chromosome complement of *Gossypium herbaceum* L. is derived from the basic number 7. In support of his assumption he points out that two of the somatic chromosomes are longer than the rest and that secondary chromosome association and

[1] Received for publication July 12, 1935; issued February 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 1069.

quadrivalent chromosomes occasionally occur during meiosis. He states (*3, p. 63*):

> The origin of the genus *Gossypium* appears to have involved tetraploidy on a basis of 7 followed by fusion of two pairs of chromosomes into one pair, thus giving $n=13$ as the fundamental number for the genus. From these so-called "diploid Asiatic cottons", which are really modified tetraploids, have arisen the so-called "tetraploid American cottons", which are phylogenetically modified octoploids. The "tetraploid" American cottons have not necessarily a common ancestor. Amphidiploidy has probably occurred in the different hybrids which result from crosses between various related ancestral diploid species. In this way the American cottons, with $2n=52$, may have arisen from separate ancestors.

Baranov (*1*) found chromosome distinctions within the somatic complement of both Asiatic and cultivated American species. Skovsted (*24*) reports that half of the chromosomes of the cultivated American species are small and the other half larger. The Asiatic and the Australian species have chromosomes comparable in size to the larger ones occurring in the cultivated American species, and the small chromosomes of the latter species are of the same size as those found in wild American species. Skovsted further records that 2 of the chromosomes of the cultivated American species and 4 of those of the Asiatic species have satellites.

The double chromosome number found in cultivated American species suggested to Longley (*19*) a duplication of the chromosomes of an ancestral type. Longley believes that his assumption is supported by the breeding results obtained in Asiatic and cultivated American species (cf. Matsuura, *20*). Longley also suggests that the high chromosome number of the cultivated American species possibly arose through hybridization. Five species, *Gossypium stocksii* Mast. (wild Asiatic), *G. sturtii* (Australian), *G. davidsonii* Kellogg (wild American), *G. harknessii* Brandeg. (wild American), and *Thurberia thespesioides*, are listed by Longley as probably most closely representing the ancestral type of species involved in the origin of cultivated American species. The occurrence of quadrivalent chromosomes and apparently of secondarily paired bivalents in cultivated American species led the writer (*27*) to support Longley's suggestion.

In general, interspecific cultivated American-Asiatic hybrids have been reported to form 13 univalent and 13 bivalent chromosomes during the first meiotic division (*1, 21*). Skovsted (*24*), however, records the frequent occurrence of polyvalents, with a conjugation as great as hexavalents. He also points out that in a triploid Asiatic-cultivated American hybrid ($3n=52$) the meiotic chromosome conjugation is the same as that found in the triploid Asiatic hybrid, except for the addition of 13 univalent chromosomes. This chromosome behavior and the fact that at least 13 univalents are always present in both $2n$ and $3n$ hybrids between cultivated American and cultivated Asiatic species led Skovsted to conclude that the pairing in the $2n$ hybrid was due to allosyndesis.

On the basis of the two groups of chromosomes found in the cultivated American species, together with the type of pairing (allosyndesis) in cultivated American-Asiatic hybrids, Skovsted concludes that the cultivated American species are allopolyploids. He suggests that one of the parental species was an Asiatic cotton or closely allied type, while the other was probably a wild American species characterized by 13 smaller chromosomes.

In a recent note the writer (*28*) set forth the essential cytological features of several interspecific hybrids in cotton. Since the cytological behavior of these hybrids bears upon the relationships disclosed in the preceding paragraphs, it appears desirable to give a more detailed account of their behavior. Additional data from new hybrids give further indications of phylogenetic relationships.

PARENTAL SPECIES AND VARIETIES

The plants used for this study were from the collection of living plants of the tribe Hibisceae of the family Malvaceae at the Rubidoux Laboratory, Riverside, Calif. Although numerous additional species and varieties were cross-pollinated, only the parents of crosses that yielded F_1 plants are listed.[3]

Cultivated American group ($n=26$)
- *Gossypium hirsutum* L. (723)[4]
  - commercial vars. Acala (W 24) and Rowden (W 12)
- *G. contextum* Cook and Hubbard (542)
- *G. punctatum* Schum. and Thon. (437)
- *G. schottii* Watt (672)
- *G. barbadense* L. (W 5)
  - commercial var. Pima (W 23)

Wild American group ($n=13$)
- *G. davidsonii* Kellogg (101)
- *G. harknessii* Brandeg. (861)
- *G. armourianum* Kearney (867)

Cultivated Asiatic group ($n=13$)
- *G. arboreum* L.
  - Taxonomic vars. *sanguineum* Watt (787) and *neglectum* Watt (785)
- *G. africanum* Watt (419)
- *G. herbaceum* L. (743)

Wild Australian species ($n=13$)
- *G. sturtii* F. Muell. (632)

Wild Arizona species ($n=13$)
- *Thurberia thespesioides* A. Gray (112)

The species and varieties listed are described by Watt (*26*), Oakley (*22*), Cook and Hubbard (*2*), Harland (*10*), and Kearney (*12*, *15*, *16*). Their meiotic chromosome behavior is described by Webber (*27*) and Longley (*19*).

INTERSPECIFIC HYBRIDIZATION

METHODS

In general, the method employed in making cross-pollinations was similar to that described by Kearney and Porter (*17*). The flowers of the pistillate parent were emasculated the evening before anthesis and were enclosed in bags. The same evening, the flowers of the staminate parent were prevented from opening by means of a spirally twisted wire. The next morning, immediately after anthesis, cross-pollinations were made. In the majority of cases the latter process was accomplished before untreated flowers of the staminate parent were completely expanded. Immediately after pollination the protective bags were replaced. These bags were removed approximately 30 hours later, at which time the stigma is apparently no longer receptive.

[3] In view of the confusion prevailing in the taxonomy of the cultivated cottons, it is not assumed that all of the species listed here are valid.

[4] The numbers in parentheses following the names of the species and varieties are those under which the seeds are cataloged by the Division of Cotton and Other Fiber Crops and Diseases, as explained by Webber (*27*).

In order to increase the percentage of successful cross-pollinations, several cotton breeders have developed special methods. These methods mainly involve the application of chemical solutions to the stigma or the treatment of the stigma with extracts of pollen, stigmas, and petals. The writer has tried several of the most promising methods and in general found that the application of chemicals or extracts to the stigma did not materially increase the percentage of successful cross-pollinations. The reported success obtained by such methods (*5*) is undoubtedly partially due to the development of parthenocarpic capsules. In cross-pollinations involving certain species and forms of cotton, the development of capsules which prove to be nothing more than empty shells is quite characteristic.

Doak (*6*) describes a method of emasculating cotton flowers which has proved very successful. It involves the splitting of the staminal column with the finger nail and pulling off the entire corolla and androecium in a single piece. This method leaves the pistil completely exposed and prevents it from being ruptured during normal shedding of the corolla. The method undoubtedly makes the stigma receptive to pollen over a longer period and prolongs the period of pollen-tube growth. The method was first suggested by Zaitzev (*29*) and has been successfully employed by the writer for several years.

Data given in table 1 indicate that the percentage of successful cross-pollinations depends on the time of day when the pollinations are made. The higher percentage of successful cross-pollinations obtained between 8:30 and 10:30 a. m. was undoubtedly due to the use of fresh pollen. In certain cotton species Kearney (*13*) has shown that the viability of the pollen "increases rapidly between 8 and 9 a. m., and shows a gradual decline after midday." Other data given in table 1 show an increase in the percentage of successful cross-pollinations in late summer, which may be due either to weather changes or to a physiological change within the pistillate parent.

TABLE 1.—*Results of cross-pollinations in cotton made (1) in the morning and in the afternoon and (2) early and late in the season*

Time of cross-pollination	Total cross-pollinations	Successful cross-pollinations		Time of cross-pollination	Total cross-pollinations	Successful cross-pollinations	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
8:30–10:30 a. m.	215	28	13.03	May 15–July 31	186	17	9.14
1–3 p. m.	100	8	8.00	Aug. 1–Sept. 30	129	19	14.73

In table 2 is given the number of cross-pollinations made within or among the five groups of species of *Gossypium*. In each of the combinations, except *Thurberia thespesioides* × *Gossypium sturtii*, several species of one or both groups were involved. The table also gives the percentage of successful cross-pollinations, the number of seeds obtained, and the percentage of germination of the seeds of cross-pollinated F_1 plants.

Table 2 shows that in most of the combinations cross-pollination was fairly successful. The correlation between the degree of relationship of the species involved and the success of cross-pollination is discussed later.

TABLE 2.—*Results of cross-pollinations within and among the five groups of species of Gossypium*

Combination	Total cross-pollinations	Successful cross-pollinations	Seeds from cross-pollinations	Germination [1] of seeds from cross-pollinations
	Number	*Percent*	*Number*	*Percent*
Cultivated American × cultivated American	16	50.00	164	90
Cultivated American × *G. sturtii*	11	18.18	38	60
Wild American × *G. sturtii*	20	30.00	63	58
Asiatic × *G. sturtii*	25	12.00	10	(2)
Cultivated American × wild American	27	18.52	56	70
Asiatic × wild American	17	5.89	5	(3)
Wild American × wild American	5	20.00	13	80
Asiatic × Asiatic	36	11.11	49	70
Cultivated American × Asiatic	125	3.19	6	(4)
Cultivated American × *Thurberia thespesioides*	23	4.35	2	100
T. thespesioides × *G. sturtii*	10	10.00	11	50
Total or average	315	11.43	417	41

[1] Except in cases otherwise noted, this percentage is based upon 10 seeds placed between moist cotton at 28° C.
[2] 4 seeds tested; no germination.
[3] 2 seeds tested; no germination.
[4] 1 seed tested; no germination.

DESCRIPTION OF HYBRIDS

The following interspecific hybrids have been grown to maturity:

Hybrids within the cultivated American group ($n=26$):
W 47.—*Gossypium hirsutum* (W 24) × *G. punctatum* (437)
W 50.—*G. hirsutum* (723) × *G. barbadense* (W 23)
W 51.—*G. contextum* (542) × *G. schottii* (672)
W 46 and W 49.—*G. barbadense* (W 23) × *G. schottii* (672)
W 54 and W 53.—*G. barbadense* (W 5) × *G. punctatum* (437)

Hybrids within the cultivated Asiatic group ($n=13$):
W 45.—*G. arboreum* var. *sanguineum* (787) × *G. africanum* (419)
W 48.—*G. herbaceum* (743) × *G. arboreum* var. *neglectum* (785)

Hybrids within the wild American group ($n=13$):
W 41.—*G. harknessii* (861) × *G. armourianum* (867)

Hybrids between the cultivated American group ($n=26$) and the wild American group ($n=13$):
W 43 and W 34.—*G. hirsutum* (W 24) × *G. armourianum* (867)
W 56.—*G. contextum* (542) × *G. armourianum* (867)
W 38 and W 36.—*G. barbadense* (W 5) × *G. harknessii* (861)

Hybrids between the cultivated American group ($n=26$) and the wild Australian species ($n=13$):
W 57 and W 33.—*G. barbadense* (W 5) × *G. sturtii* (632)

Hybrids between the wild American group ($n=13$) and the wild Australian species ($n=13$):
W 39, W 52, and W 35.—*G. sturtii* (632) × *G. harknessii* (861)
W 40.—*G. sturtii* (632) × *G. armourianum* (867)
W 59.—*G. davidsonii* (101) × *G. sturtii* (632)

Hybrids between *Thurberia* ($n=13$) and the wild Australian species ($n=13$):
W 58.—*Thurberia thespesioides* (112) × *Gossypium sturtii* (632)

The following interspecific hybrids did not reach maturity:

Hybrids between the cultivated American group ($n=26$) and *Thurberia* ($n=13$):
W 44.—*Gossypium hirsutum* (W 12) × *Thurberia thespesioides* (112)

With the exception of *Gossypium hirsutum* × *Thurberia thespesioides* all of these F_1 hybrids flowered freely. The hybrids between species within a group are self-fertile and produce seed when fecundated with pollen of either parental species; also, both parental species are fertile

with the hybrid pollen. Such hybrids exhibit considerable hybrid vigor. The hybrids between species of different groups, on the contrary, are perfectly sterile and produce no seed when backcrossed to either parent. The parents also are sterile to the hybrid pollen. With the exception of *G. hirsutum* × *T. thespesioides* all of these hybrids are exceedingly vigorous.

In several instances, the individuals of a hybrid between a pair of species exhibited variation in certain characters. Since other hybrids involving one or the other of the same parental species exhibited similar variations, it may be supposed that the common parent was heterozygous. In order to prevent repetition and complications in the following descriptions, such variations and numerous minor characteristics of the hybrids are not described.[5]

Gossypium hirsutum × *punctatum*.—Plants *hirsutum*-like in all characters, sparsely hirsute on the young stems and petioles. Petals very pale yellow, anthers pale yellow as in *punctatum*. Bolls with 4 to 5 locules, large, nearly pointless, with or without very short apical furrows. Growth habit somewhat prostrate.

Gossypium hirsutum × *barbadense*.—Typical of this combination in F_1. Petals large, martius yellow, with rather faint, striate spots. Pollen empire yellow. Bolls large, somewhat pointed, smooth.

Gossypium contextum × *schottii*.—Stems, petioles, leaf veins, and involucel very dark purple. Leaf blades deeply and narrowly lobed. Petals spotless, lighter than martius yellow. Anthers approximately empire yellow. Bolls with four locules, somewhat taper-pointed, very smooth, with short but pronounced apical furrows.

Gossypium barbadense × *schottii*.—Plants glabrous or nearly so, except on very young parts. Stems dark purple. Leaf blades deeply and narrowly five-lobed. Calyx with merely undulate margin and many oil glands. Petals pale green-yellow, distinctly spotted. Pollen between empire yellow and lemon-chrome. Bolls mostly with four locules, pointed, rather deeply pitted, with short but pronounced apical furrows.

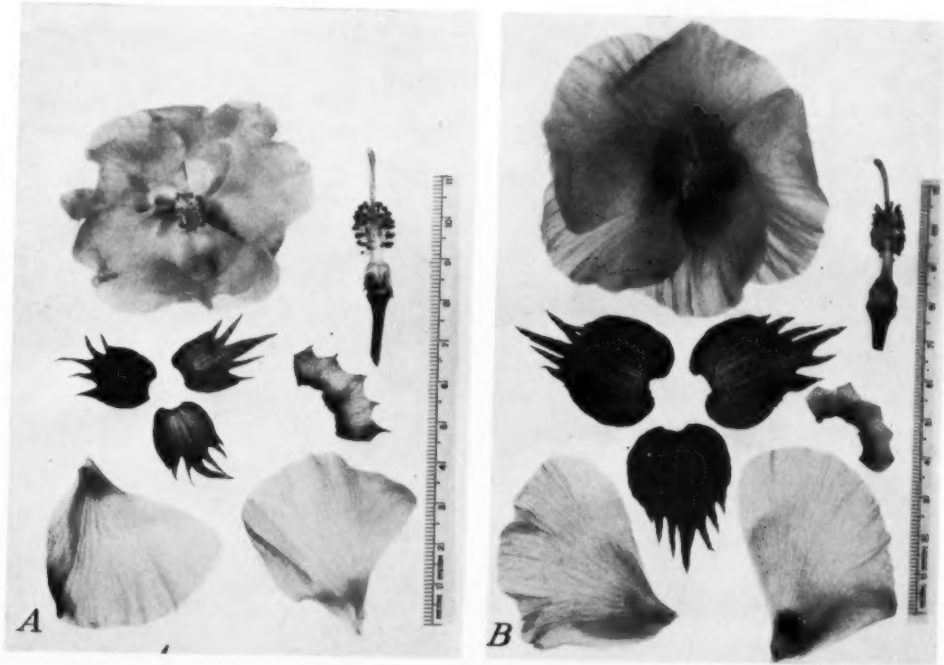
Gossypium barbadense × *punctatum*.—Plants much as in *G. hirsutum* × *barbadense* F_1, nearly glabrous except on the very young parts. Petals approximately martius yellow, with rather faint striate spots. Pollen near empire yellow. Bolls with 3 to 5 locules, their surface smooth and light colored.

Gossypium arboreum var. *sanguineum* × *africanum*.—The characters of both parents are about equally represented in this hybrid. Young stems, petioles, and peduncles very hirsute. Older stems dark brown. Leaf blade more like that of *sanguineum*, deeply lobed, with relatively narrow acuminate lobes. Involucels like *sanguineum* in having bractlets of more triangular shape but deeply laciniate with setose-tipped teeth as in *africanum*. Petals ruffled on the outer edge, nearly as large as in *africanum*, approaching *sanguineum* in color (pomegranate-purple), but shading to yellowish around the spot rather than, as in *sanguineum*, to whitish. Spot intense bordeaux color. Anthers near xanthine-orange. Bolls mostly with three locules, oblong-ovoid, short-pointed, smooth, reddish where exposed.

Gossypium herbaceum × *arboreum* var. *neglectum*.—Older stems nearly black; twigs, petioles, and peduncles hirsute. Leaf blades much nearer *neglectum* in shape. Involucel resembling *neglectum* in its more triangular bractlets with slenderer, more setose teeth, the bractlets connate near base. Calyx like *herbaceum* in its undulate or very short-dentate margin, the teeth deltoid and not, as in *neglectum*, setose-tipped. Petals approaching *neglectum* in size and shape and in the larger size of the very intense spot; between martius yellow and picric yellow, the spot between carmine and ox-blood red. Anthers light cadmium. Bolls mostly with four locules, short-ovoid, very plump, abruptly pointed, with long, deep apical furrows, red where exposed.

Gossypium harknessii × *armourianum*.—Plants more open and with more ascending branches than in either parent, obscurely puberulent on the very young parts, soon glabrous. Twigs light mahogany colored, older bark light

[5] The following descriptive notes were supplied mainly by T. H. Kearney. The colors were determined by comparison with the standards in the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.



Typical F_1 flowers and flower parts: A, *Gossypium hirsutum* × *armourianum;* B, *G. barbadense* × *harknessii.*

Gossypium barbadense × *stru̇tii*. Typical F_1 flower and flower parts. The petals are rose red in color; in the parents they are yellow and pale mauve respectively.

brown. Leaf blades grayish green (yellowish in *harknessii*, deeper green in *armourianum*), less shiny above than in *armourianum*, nearer *harknessii* in size and shape, entire to very shallowly three-lobed with rounded lobes, broadly ovate, obtuse or acutish, rather deeply cordate. Involucel light green, resembling that of *harknessii* in its large, oblong-ovate bractlets, which are persistent until anthesis. Petals approaching those of *harknessii* in size and shape, pale viridine yellow. Bolls mostly with three locules, broadly ovoid.

Gossypium hirsutum × *armourianum*.—Plants glabrous except on the very young parts, large, woody, many-stemmed but open, the branches wide-spreading, with long internodes, the branchlets conspicuously zigzag. Leaf blades light green with a small brown pulvinus, mostly shallowly three-lobed. Involucel persistent after anthesis; bractlets separate, with a few long teeth. Calyx dentate, sometimes with deltoid-setose teeth nearly as long as the height of the undivided portion and having very numerous oil glands. Petals pale green-yellow or martius yellow, reddish on the exposed edges; spot when present [6] faint and striate to rather intensely pomegranate-purple. Filaments colorless, or the lowest purple. Anthers with or without red color on the connective. Pollen light cadmium. Exserted portion of the pistil very long. Plate 1, *A*, shows characteristic flower parts of this hybrid.

Gossypium contextum × *armourianum*.—Closely similar in all characters to *G. hirsutum* × *armourianum*.

Gossypium barbadense × *harknessii*.—The plants show a predominance of *barbadense* characters. Stems decidedly woody, twigs reddish brown. Petioles less densely puberulent than in *harknessii*, with longer hairs. Leaf blades *barbadense*-like but smaller, the larger ones deeply five-lobed, puberulent with short, stellate hairs, with a deeper-colored pulvinus than in *barbadense* and with a small deltoid or lanceolate nectary on the midvein toward base. Involucel large, showing no sign of falling at anthesis (a character of *harknessii*); bractlets deeply laciniate with broad teeth, separate or nearly so. Calyx ciliolate, with undulate margin and numerous black oil glands. Petals large, pale green-yellow in W 36, between martius yellow and picric yellow in W 38, the spots large but rather faint. Filaments purplish. Anthers light cadmium. Pistil with the length of the exserted portion equaling or surpassing that of the staminiferous portion of the column. Bolls (5 weeks old, parthenocarpic) resembling those of *harknessii* in shape and in the reddish-brown oil glands. A characteristic plant and characteristic flower parts of this hybrid are shown in figure 1 and in plate 1, *B*.

Gossypium barbadense × *sturtii*.—Plants glabrous or nearly so, resembling *sturtii* in their rather stiff habit, with long, nearly erect, vegetative branches. Fruiting branches with very long internodes. Young bark reddish, glaucous. Leaf blades not glaucous, varying on different plants from deeply and rather narrowly five-lobed to much more shallowly and broadly three-lobed, with large lanceolate or deltoid nectaries near the base of the midvein or of all three principal veins. Involucel glabrous, one-half to two-thirds as high as the corolla; bractlets separate to the base, more or less deeply laciniate with few or rather numerous subulate, setose-tipped teeth. Calyx ciliolate, with numerous oil glands, sharply dentate, with deltoid-subulate teeth. Petals densely ciliate toward base, between Tyrian pink and rose colored (yellow in *barbadense*, pale mauve in *sturtii*) with very large, intense, feathered spots of *sturtii* character and varying from pomegranate-purple to bordeaux in color. Column very long and staminiferous nearly to its base, as in *sturtii*. Filaments purple. Anthers reddish. Pollen yellow. Pistil long-exserted, but the exserted portion much shorter than the staminiferous part of the column. Figure 2 shows a characteristic plant of *G. barbadense* × *sturtii*, and plate 2 shows characteristic flower parts.

Gossypium sturtii × *harknessii*.—Plants of intermediate habit, less stiff and erect than *sturtii*, but much less spreading than *harknessii*, more open than either parent, with numerous erect or ascending branches, nearly glabrous. Petioles obscurely puberulent toward apex. Leaf blades glaucescent, nearer *harknessii* in color, broadly deltoid, entire to rather deeply three-lobed, subcordate, acuminate, the lateral lobes rounded or acute, with a very small, slit-like nectary near the base of the midvein. Involucel more persistent than in *harknessii*, larger than in either parent, glabrous; bractlets widely separate, ovate or lance-ovate, setose-acuminate, narrowed at base, usually undulate or sparsely denticulate on the margin, but sometimes dentate with very few, short or long,

[6] The *armourianum* parent was presumably heterozygous for this character.

subulate-setose teeth. Calyx very thin, obscurely ciliolate, dentate with short, deltoid, very acute teeth and with numerous oil glands. Petals of a rather dingy color between rose-pink and hellebore-red, with a broad, pinkish-buff stripe on the exposed edge, the spot large, much like that of *sturtii* in shape but less feathered, bright carmine. Column often very long, as in *sturtii*. Filaments much shorter than in *harknessii;* anthers between dragon-blood red and Etruscan red. Pollen pale yellow. Pistil with the exserted portion long but usually shorter than the column. Figure 3 shows a characteristic plant of *G. sturtii* × *harknessii*.

FIGURE 1.—*Gossypium barbadense* × *harknessii*. Typical F_1 plant.

Gossypium sturtii × *armourianum*.—No open flowers have been produced. Plants minutely puberulent on the very young parts, soon glabrous, low, compact, with very numerous, ascending branches, much lower and more spreading than in *sturtii* but with fewer and less spreading branches than in *armourianum*. Leaf blades not glaucous and of about the same color as in *armourianum*, broadly ovate, subcordate, abruptly short-acuminate with a cartilaginous tip, usually with a small slitlike nectary near the base of the midvein. Involucel of intermediate size and character, more persistent than in *armourianum;* bractlets widely separate, narrowly lanceolate, entire, or occasionally with a minute tooth near apex, very sharply cartilaginous-acuminate. Calyx conspicuously dentate with deltoid-subulate teeth longer and slenderer than in *armourianum* and having numerous, conspicuous, prominent black oil glands.

Gossypium davidsonii × *sturtii*.—Plants very open, with few ascending branches, obscurely puberulent toward the apex of the petiole, on the pulvinus, and near the base of the leaf veins, otherwise glabrous or very nearly so. Leaf blades glaucous but much less so than in *sturtii*, all entire or occasionally with a very short tooth, broadly ovate, short-acuminate, subcordate. Involucel nearly intermediate, obscurely ciliolate, otherwise glabrous; bractlets quite separate, oblong-ovate, with few short, deltoid, setose-tipped teeth. Calyx dentate with deltoid, obtuse to setose-acuminate teeth and with fairly numerous oil glands.

FIGURE 2.—*Gossypium barbadense* × *sturtii*. Typical F_1 plant.

Corolla small. Petals pale rhodonite pink, the spot *sturtii*-like, pomegranate-purple. Column much as in *sturtii*, long, the staminiferous portion longer than the exserted part of the long-exserted pistil. Filaments short, purplish. Anthers pale orange-yellow, drying pinkish. Pollen pale yellow. Figure 4 shows a characteristic plant of *G. davidsonii* × *sturtii*, and plate 3, *A*, shows characteristic flower parts.

Gossypium hirsutum × *Thurberia thespesioides*.—The plants died soon after the seedling stage. Leaf blade much like that of *Thurberia* in shape, ovate-lanceolate, deeply and narrowly lobed.

Thurberia thespesioides × *Gossypium sturtii*.—Plants much more open in habit than *G. sturtii*, with numerous nearly erect limbs; young branches somewhat

glaucous. Leaf blades green, slightly glaucous, much like those of *Thurberia* in shape, ovate-lanceolate and entire to deeply and narrowly three-lobed; lobes long-acuminate, setose at apex as in *G. sturtii*, with an elongate slitlike nectary very near the base of the midvein. Involucel with bractlets widely separate, as in both parents; bractlets lanceolate, intermediate in width but near *G. sturtii* in length, setose-tipped, often with 1 or 2 setose teeth. Calyx abruptly dentate with subulate-setose teeth. Petals approaching those of *G. sturtii* in size and shape, in color intermediate between white and pale amaranth-pink, fading to the latter color, the spot between pomegranate-purple and bordeaux, very like that of *G. sturtii*. Column much as in *G. sturtii*, elongate, with short filaments,

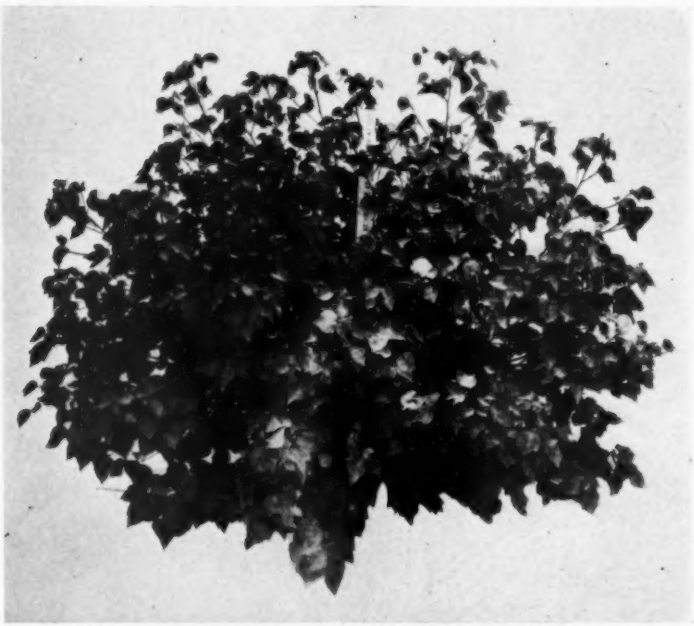


FIGURE 3.—*Gossypium sturtii* × *harknessii*. Typical F_1 plant.

but with fewer stamens and these not extending so near the base of the column. Filaments purple. Anthers daphne pink. Pistil, with the exserted portion long but not equaling the column. Plate 3, *B*, shows characteristic flower parts of *T. thespesioides* × *G. sturtii*.

CYTOLOGICAL TECHNIQUE

Cytological material was collected in full sunlight between May and September. In order to insure characteristic meiotic behavior, weekly checks were made with material from several individuals. Since these individuals continued throughout the season to exhibit the same cytological behavior, the data herein reported may be assumed to be characteristic of all representatives of a given combination.

The study was limited to pollen mother cells and sporads from anthers fixed in Carnoy's fluid 10 to 30 minutes, washed and preserved in alcohol, and crushed in iron-acetocarmine. The use of Carnoy's fluid and alcohol previous to crushing in acetocarmine is of advantage

Typical F_1 flowers and flower parts: A, *Gossypium davidsonii* × *sturtii*; B, *Thurberia thespesioides* × *G. sturtii*.

in that the extremely soft pollen mother cells of *Gossypium* are hardened and much of the matter obscuring the chromosomes is removed from them. Acetocarmine preparations have the distinct advantage of keeping pollen mother cells in a solution. Hence, the cells can be moved to allow vision at different angles and can be easily crushed to allow a more detailed study.

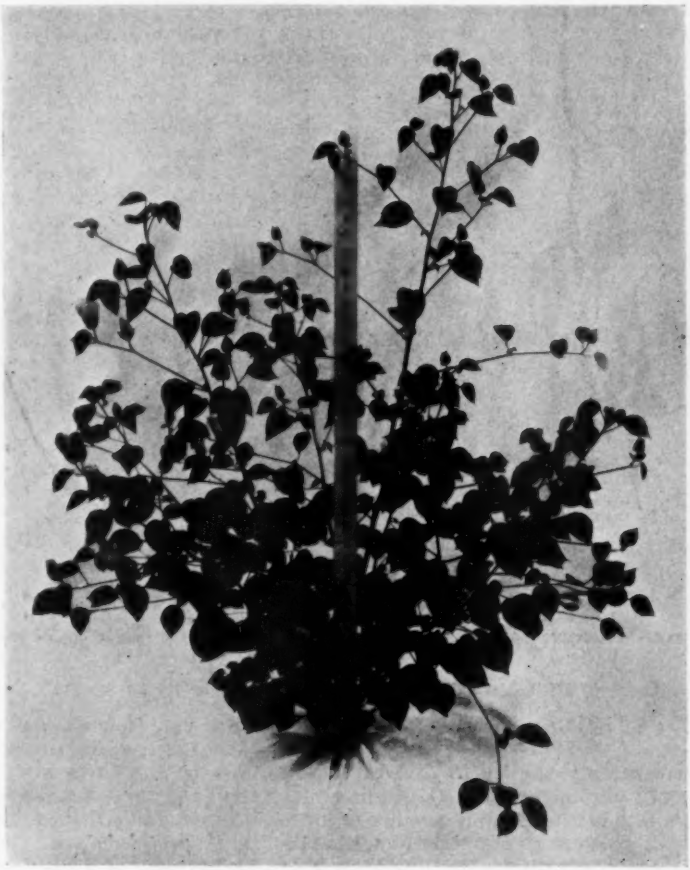


FIGURE 4.—*Gossypium davidsonii* × *sturtii*. Typical F_1 plant.

All drawings were made at bench level with a camera lucida having a 1.5-mm objective (1.3 N. A.), an 18 × compensating ocular, and a tube length of 160 mm, giving a magnification of 4,650 diameters.

MEIOTIC BEHAVIOR OF F_1 PLANTS

HYBRIDS BETWEEN CULTIVATED AMERICAN SPECIES (N 26 × N 26)

The reduction divisions of the hybrids between species within the cultivated American group of cottons, listed on page 1051, are similar

to those of their parents (*27*). Although there are generally 26 bivalent chromosomes at first metaphase, occasionally 1 or 2, and rarely 3 quadrivalent chromosomes are formed. The bivalent partners are united at one or both ends by 1 or 2 or rarely 3 chiasmata. The total number of chiasmata is apparently somewhat smaller than in 26-paired species. Figure 5 depicts a characteristic first metaphase of *Gossypium barbadense* × *schottii*.

During the first anaphase no irregularities occur and the homotypic divisions are nearly normal. Like the parental species, approximately 4 percent of the sporads contain microcytes.

FIGURE 5.—*Gossypium barbadense* × *schottii*. Lateral view of first metaphase in a slightly crushed pollen mother cell, showing 26 bivalent chromosomes.

HYBRIDS BETWEEN ASIATIC SPECIES (N 13 × N 13)

The meiotic divisions of *Gossypium arboreum* var. *sanguineum* × *africanum* and of *G. herbaceum* × *arboreum* var. *neglectum* also present a picture very similar to that of their parents (*27*). There are 13 bivalent chromosomes. At the first metaphase the total number of chiasmata is considerably smaller than that occurring in either parent and all chiasmata are strictly terminal. In *G. arboreum* var. *sanguineum* × *africanum* the union between the partners of 2 bivalent chromosomes is apparently rather weak, and in several cases 2 univalent chromosomes have been observed. In the anaphase of the latter hybrid occasionally the univalents lag and rarely are left in the plasma. Although in both hybrids the majority of second metaphase plates contain 13 chromosomes, plates with 12 and 14 are not uncommon. Approximately 3 percent of the sporads of *G. herbaceum* × *arboreum* var. *neglectum* and 6 percent of those of *G. arboreum* var. *sanguineum* × *africanum* exhibit microcytes.

HYBRIDS BETWEEN WILD AMERICAN SPECIES (N 13 × N 13)

The meiotic divisions of *Gossypium harknessii* × *armourianum* are similar to those of both parents (*27*). During diakinesis and the first metaphase there are 13 pairs of chromosomes. Both the first and second divisions are regular and the tetrads are normal. Figure 6 depicts a characteristic first metaphase.

FIGURE 6.—*Gossypium harknessii* × *armourianum*. Profile view of first metaphase in a pollen mother cell, showing 13 bivalent chromosomes.

HYBRIDS BETWEEN CULTIVATED AMERICAN AND WILD AMERICAN SPECIES (N 26 × N 13)

During the first metaphase in *Gossypium hirsutum* × *armourianum* and *G. contextum* × *armourianum* there are usually 13 bivalent and 13 univalent chromosomes. Among 43 pollen mother cells of *G. hirsutum* × *armourianum*, 4 formed 1 quadrivalent, 11 bivalent, and 13 univalent chromosomes; and 1 formed 2 quadrivalent, 9 bivalent, and 13 univalent chromosomes. The bivalents form a well-organized equatorial plate which generally contains a few elongated univalents. The remaining univalents are more or less spherical and are scattered

FIGURE 7.—*Gossypium hirsutum* × *armourianum*. Lateral view of first metaphase in a pollen mother cell, showing 12 bivalent and 15 univalent chromosomes.

over the achromatic figure. The majority of bivalent partners are united at one end by 1 or 2 terminal chiasmata. In some bivalents the union between partners is comparatively poor and rarely it is ineffectual. In the latter case 2, 4, or 6 additional univalent chromosomes occur. Figure 7, which illustrates the condition at the approach of the heterotypic anaphase, depicts 12 bivalent and 15 univalent chromosomes.

At the first anaphase the bivalent partners are distributed normally and the scattered univalents pass toward adjacent polar regions. The univalents in the equatorial region become laggards and elongate as though about to divide. They are later distributed at random toward the poles, but on account of their delayed movement they are frequently left in the plasma. Occassionally a univalent completes division and the resulting parts are left in the plasma or included in the daughter nuclei. Since the products of such division are of various sizes, the division is undoubtedly a fragmentation.

The homotypic divisions are fairly regular. There are usually two major and several diminutive achromatic figures. Although the majority of chromosomes divide normally, laggards are not uncommon. At the completion of meiosis highly abnormal sporads occur, which contain from 2 to 12 spores of various sizes.

The heterotypic divisions of *Gossypium barbadense* × *harknessii* differ slightly from those of *G. hirsutum* × *armourianum*. In no case have more than 13 univalent chromosomes been observed. In *G. barbadense* × *harknessii* it is apparent that more of the bivalent chromosomes are united at both ends than in hybrids between *G. hirsutum* or *G. contextum* and *G. armourianum*. The majority of pairs are more or less ring-shaped and several exhibit three chiasmata. It is also apparent that the unions between bivalent partners are comparatively stronger than those occurring in *G. hirsutum* × *armourianum*.

HYBRIDS BETWEEN CULTIVATED AMERICAN AND WILD AUSTRALIAN SPECIES (N 26 × N 13)

The meiotic divisions of *Gossypium barbadense* × *sturtii* are very irregular. In the heterotypic division there is so much variation that in many pollen mother cells the type of conjugation could not be accurately determined. The following data for the first metaphase chromosome conjugation in 45 pollen mother cells, however, are believed to be accurate and characteristic of the majority of pollen mother cells.

Type of conjugation[1]	*Number of pollen mother cells*
$0_{II}+39_{I}$	25
$1_{II}+37_{I}$	8
$2_{II}+35_{I}$	5
$3_{II}+33_{I}$	5
$4_{II}+31_{I}$	2

An examination of the foregoing data shows that from 0 to 4 bivalent and from 31 to 39 univalent chromosomes are formed. Although the bivalents lie in the equatorial region, they do not form a definite plate. Their partners are united by a single chiasma, which often appears imperfect. In the majority of cases, the univalents are uniformly scattered over the achromatic figure. Figure 8 depicts a first metaphase having 2 bivalent and 35 univalent chromosomes.

The remaining stages of meiosis are similar to those found in *Gossypium hirsutum* × *armourianum*. In *G. barbadense* × *sturtii*, however, the irregularities are of greater magnitude. Also in the latter hybrid several somatic (39-chromosome) second-metaphase plates have been observed. At the completion of meiosis the sporads con-

[1] The subscripts I and II are cytological symbols designating univalent and bivalent chromosomes.

tain from 2 to 14 spores, which vary in size from very small ones to those nearly twice the size of the tetrad spores of the *barbadense* parent.

HYBRIDS BETWEEN WILD AMERICAN AND WILD AUSTRALIAN SPECIES (N 13 × N 13)

The types of conjugation at first metaphase in *Gossypium sturtii* × *armourianum*, *G. sturtii* × *harknessii*, and *G. davidsonii* × *sturtii* are

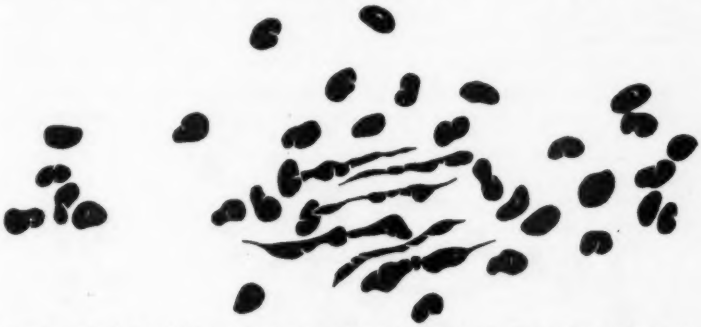


FIGURE 8.—*Gossypium barbadense* × *sturtii*. Profile view of first metaphase in a pollen mother cell, showing 2 bivalent and 35 univalent chromosomes. Four of the univalent chromosomes are in process of fragmentation.

given in table 3. Although chromosome pairing is variable in these hybrids, they exhibit a larger number of pairs than does *G. barbadense* × *sturtii*, and pairing is more frequent. Table 3 indicates that from 0 to 6 bivalent and from 14 to 26 univalent chromosomes are formed in hybrids between the wild American species and *G. sturtii*. The arrangement of bivalent and univalent chromosomes on the achro-

FIGURE 9.—*Gossypium sturtii* × *armourianum*. Profile view of first metaphase in a pollen mother cell, showing 6 bivalent and 14 univalent chromosomes. Three of the univalent chromosomes are undergoing fragmentation.

matic figure is similar to that recorded in the case of *G. barbadense* × *sturtii*. Also, like the latter hybrid, the bivalent partners are united at one end by a single chiasma, which is comparatively imperfect. Figure 9 depicts a first metaphase of *G. sturtii* × *armourianum* having 6 bivalent and 14 univalent chromosomes; 3 of the latter are apparently fragmenting. Figure 10 shows a first metaphase of *G. davidsonii* × *sturtii* having 26 chromosomes.

TABLE 3.—*First-metaphase chromosome conjugation in pollen mother cells of hybrids between Gossypium sturtii and wild American species*

Hybrid	Pollen mother cells with indicated type of conjugation						
	$0_{II}+26_{I}$	$1_{II}+24_{I}$	$2_{II}+22_{I}$	$3_{II}+20_{I}$	$4_{II}+18_{I}$	$5_{II}+16_{I}$	$6_{II}+14_{I}$
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
G. sturtii × *armourianum*	26	13	4	3	----------	1	2
G. sturtii × *harknessii*	11	4	1	1	1	----------	----------
G. davidsonii × *sturtii*	17	5	4	3	----------	2	----------

The irregularities occurring in the remaining stages of meiosis are similar to those exhibited by *Gossypium hirsutum* × *armourianum*,

FIGURE 10.—*Gossypium davidsonii* × *sturtii*. Profile view of first metaphase in a pollen mother cell, showing 26 univalent chromosomes, 5 of which are undergoing fragmentation.

but are somewhat smaller and considerably less pronounced in these hybrids than in *G. barbadense* × *sturtii*. Although the sporads of all three hybrids in this group are generally highly abnormal, those of *G. sturtii* × *armourianum* most nearly approach the tetrad.

FIGURE 11.—*Thurberia thespesioides* × *Gossypium sturtii*. Profile view of first metaphase in a pollen mother cell, showing 26 univalent chromosomes, 5 of which are fragmenting.

HYBRIDS BETWEEN THURBERIA THESPESIOIDES AND GOSSYPIUM STURTII (N 13 × N 13)

During the first metaphase of *Thurberia thespesioides* × *Gossypium sturtii* there is no visible chromosome pairing. The 26 univalent chromosomes are scattered over the achromatic figure. Now and then several long slender univalents are found in the equatorial region. Although these latter univalents occasionally divide, they usually exhibit a random distribution along with the remaining univalents. The homotypic divisions are quite regular, even though a few chromosomes often lag. The sporads are highly abnormal. Figure 11 depicts a characteristic first metaphase of *T. thespesioides* × *G. sturtii*.

A NATURAL HYBRID BETWEEN AN ASIATIC AND A CULTIVATED AMERICAN SPECIES (N 13 × N 26)

The writer has made a careful cytological study of a natural hybrid between an Asiatic and a cultivated American species of *Gossypium* ($n13 \times n26$). The particular Asiatic species to which one of the parents of this hybrid belonged is unknown. The American parent probably was *Gossypium hirsutum*. Longley (*19*) reported that this natural hybrid exhibited variable pairing, and he figured a metaphase with 14 bivalent and 11 univalent chromosomes. In the same hybrid Skovsted (*24*) reported the pairing in two pollen mother cells to be, respectively, 13 univalents, 8 bivalents, 1 tetravalent, and 1 hexavalent; and 14 univalents, 8 bivalents, 1 trivalent, and 1 hexavalent.

The writer's studies indicate that the meiotic behavior of this natural hybrid closely approaches that reported by Nakatomi (*21*) in similar hybrids and resembles the behavior of *Gossypium hirsutum* × *armourianum* reported above. Although during diakinesis there are generally 13 pairs and 13 single chromosomes, occasionally 1 to 3 quadrivalents are present. The majority of pairs are united at one end by 1 or 2 chiasmata. At least 4 pairs exhibit 3 chiasmata. Although several pairs display interstitial chiasmata, the majority have only subterminal or terminal ones. At the approach of the first metaphase the degree of terminalization of the chiasmata appears to vary. It is usually complete, but occasionally subterminal, and very rarely interstitial chiasmata occur. In several cases, the union of rod-shaped pairs is comparatively imperfect.

Following are the data for the first-metaphase chromosome conjugation in 62 pollen mother cells of this natural hybrid in F_2.

Type of conjugation	*Number of pollen mother cells*
$13_{II} + 13_{I}$	38
$12_{II} + 15_{I}$	5
$11_{II} + 17_{I}$	3
$10_{II} + 19_{I}$	3
$9_{II} + 21_{I}$	1
$1_{IV} + 11_{II} + 13_{I}$	7
$2_{IV} + 9_{II} + 13_{I}$	2
$3_{IV} + 7_{II} + 13_{I}$	1
$4_{IV} + 6_{II} + 11_{I}$	2

An examination of the foregoing data reveals that out of 62 pollen mother cells 12 contained from 1 to 4 quadrivalent chromosomes and 38 had 13 bivalent and 13 univalent chromosomes. The remaining 12 pollen mother cells displayed a decrease in the amount of pairing. As in the case of *Gossypium hirsutum* × *armourianum*, where pairing is somewhat variable, when the maximum number of bivalents are present several of them exhibit comparatively poor or imperfect unions. Since the number of bivalents exhibiting imperfect unions decreases as the number of univalents increases, there can be little doubt that such unions are occasionally ineffectual. Figure 12 depicts a first metaphase having 2 quadrivalent, 9 bivalent, and 13 univalent chromosomes.

The remaining characteristics in meiosis resemble those displayed by *Gossypium hirsutum* × *armourianum*. However, in this natural hybrid, among the highly abnormal sporads, diads are fairly common.

DISCUSSION

INCOMPATIBILITY BETWEEN SPECIES OF GOSSYPIUM

The degree of compatibility between different species is commonly supposed to correspond with their nearness of relationship, as indicated by their degree of morphological similarity. In *Gossypium* it is evident that such a supposition is not always true. Interspecific hybrids within the cultivated American group of cottons are easily obtained, while those between species within the Asiatic group are fairly difficult to obtain. Although the Asiatic and cultivated American species are not very dissimilar morphologically, hybrids between them are very difficult to obtain. On the other hand, *G. sturtii*, which is morphologically one of the most distinct species, readily hybridizes with species of all other groups of *Gossypium*.

FIGURE 12.—A natural hybrid between an Asiatic and a cultivated American cotton. Profile view of first metaphase in a slightly crushed pollen mother cell, showing 2 quadrivalent, 9 bivalent, and 13 univalent chromosomes. Two of the univalent chromosomes are in process of fragmentation.

The wild American cottons, although less dissimilar to the cultivated cottons than is *G. sturtii*, do not hybridize with them as freely as does *G. sturtii*.

NONVIABILITY OF THE GAMETES AND THE ZYGOTE IN INTERSPECIFIC HYBRIDS

In interspecific *Gossypium* hybrids the relationship of the parental species, as indicated by morphological characters, and the normality of the heterotypic division are usually correlated. In turn, the degree of fertility is correlated with the amount of chromosome pairing. It is logical to suppose that sterility in *Gossypium* hybrids is due to the production of nonviable gametes. In such hybrids as *G. sturtii* × *harknessii*, *Thurberia thespesioides* × *G. sturtii*, and *G. barbadense* × *sturtii* the parental species are distantly related and the hybrids are completely sterile. In these hybrids there is little or no chromosome pairing during the heterotypic division. The chromosomes are irregularly distributed to daughter nuclei and some are

left in the plasma. On the other hand, the cultivated American species and the wild American species are presumably more closely related than the species involved in the hybrids with *G. sturtii*. In *G. barbadense* × *harknessii* and *G. hirsutum* × *armourianum* this supposition of relationship is borne out by distinct chromosome pairing. Although one would expect a greater production of viable gametes in the latter hybrids, the plants are completely sterile. The expectation is approached only in the formation of seedless capsules.

Interspecific hybrids within the cultivated American group of cottons exhibit complete chromosome pairing and are highly fertile. Likewise, the hybrid *Gossypium herbaceum* × *arboreum* var. *neglectum*, between species of the Asiatic group, exhibits complete chromosome pairing and is highly fertile. However, the amount of chromosome pairing in the inter-Asiatic hybrid *G. arboreum* var. *sanguineum* × *africanum* is slightly reduced and the fertility is correspondingly less.

Apparently, in crosses involving *Thurberia thespesioides*, a near relative if not actually a member of the genus *Gossypium*, there is a high degree of zygote mortality. Harland and Atteck (*11*) found that crosses between *G. stocksii* (a wild Asiatic species with $n=13$) and *Thurberia* were quite easily made, but that the hybrid seedlings were weak and died after 2 or 3 leaves were formed. The same authors found a similar condition in crosses between *G. davidsonii* and *Thurberia*. However, by using as pistillate parents 2 complex American hybrids, these authors obtained 8 healthy hybrids; and no mention is made of weak plants or of mortality. In the writer's experiments 10 percent of the cross-pollinations between *Thurberia* and *G. sturtii* produced good capsules. Approximately 50 percent of the hybrid seeds germinated and 66 percent of the seedlings grew to be healthy plants. Likewise, seedlings of hybrids between *G. hirsutum* and *Thurberia* are easily obtained, but they are extremely weak and die during early stages of development.

INHERITANCE IN INTERSPECIFIC HYBRIDS

In *Gossypium* there are relatively few reported cases of monohybrid segregation. Kearney (*14*) expresses the belief that this is partly due to the fact that most investigators have considered mainly characters of economic importance, such as size of the bolls and length and abundance of the lint, which are conditioned by several factors. Another reason, suggested by Harland, is that genetic analyses have dealt largely with interspecific rather than intraspecific hybrids. Harland (*8, 9*) found that several characters which exhibited alternative inheritance in intraspecific hybrids exhibited a "quantitative" type of inheritance in interspecific hybrids. He attributes the latter behavior to modifying factors, which have the effect of obscuring the segregation of characters.

Very little is known regarding the inheritance of the characters in the interspecific hybrids under discussion. Since back-crossed or selfed generations have not been grown, genetical analyses are wanting. However, several characters show a tendency to dominance in these hybrids between species. In color, the dark-red plant body in *Gossypium arboreum* var. *sanguineum* and *G. schottii* dominates the green plant body of other species. Likewise, the pinwheel appearance of the corolla spots in *G. sturtii* appears to dominate the type of corolla

spots characteristic of other species. The narrowly lobed leaf of *Thurberia* partially dominates the entire leaf of *G. sturtii*. To a lesser degree the lobed leaf of *G. hirsutum* dominates the entire leaf of *G. armourianum*. The presence of a petal spot in *G. sturtii*, *G. barbadense*, and *G. armourianum* tends to be dominant over absence of petal spot in other species. Similarly, colored pollen is partially dominant over uncolored pollen.

Interspecific *Gossypium* hybrids derived from species having the same number of chromosomes usually exhibit an intermediate expression of most of the contrasting characters of the parents. Such a condition is probably due to the lack of dominance or to an equal number of dominant factors being contributed by each parent. On the other hand, hybrids between species having, respectively, 13 and 26 as the haploid number of chromosomes resemble the parent having 26 haploid chromosomes. This is probably due to the transmission of a greater number of dominant factors along with the larger number of chromosomes. Except for the shape of the young parthenocarpic bolls in F_1 of *G. barbadense* × *harknessii*, the *barbadense* parent appears to dominate *harknessii*. The decided resemblance of this hybrid to *G. barbadense* may be due to (1) the transmission from the *barbadense* parent of a greater number of dominant factors along with the larger number of chromosomes or (2) close genetic similarity of the *harknessii* chromosomes to 13 of the *barbadense* chromosomes (*25*). In the latter case, the *harknessii* chromosomes are sufficiently homologous to certain *barbadense* chromosomes to be combined with them without disturbing the *barbadense*-like nature of the hybrid.

PHYLOGENETIC CONCEPTIONS

Gates (*7*) has recently reviewed the literature bearing upon the origin of species in the genus *Gossypium*. He points out that both Davie and Skovsted have suggested that the species with 13 pairs of chromosomes are modified tetraploids. Gates also calls attention to the fact that the best evidence in support of this suggestion is found in the observations by Davie of secondary chromosome association in *G. herbaceum*. Neither Webber (*27*) nor Skovsted (*23*) have found secondary association within the Asiatic species. That there are no homologous chromosomes in the haploid sets of *Thurberia thespesioides* and *G. sturtii* is shown by the lack of chromosome pairing in hybrids between these two species. On the other hand, the slight amount of pairing in the hybrids between *G. sturtii* and the wild American species may indicate that certain chromosomes within the haploid set of the wild American species and of *G. sturtii* are homologous. In this case, however, it remains to be determined whether the pairing is within the wild American haploid set of chromosomes or between chromosomes of the parental species.

Skovsted (*24*) and Davie (*3*) have also suggested that the cultivated American cottons are allotetraploids. Skovsted concluded that their origin may be ascribed to the doubling of the chromosomes in a hybrid between an Asiatic and a wild American species. That the chromosomes of the wild American and of the Asiatic species are homologous to 13 of the chromosomes in the cultivated American cottons is indicated by the occurrence of 13 pairs of chromosomes in the hybrids *Gossypium hirsutum* × *armourianum*, *G. barbadense* × *harknessii*

and the natural hybrid between an Asiatic and a cultivated American species. The fact that *G. barbadense* × *sturtii* rarely exhibits chromosome pairing and never shows a high degree of conjugation indicates that the pairing which occurs in the F_1 of the hybrids *G. hirsutum* × *armourianum*, *G. barbadense* × *harknessii*, and of the natural hybrid between an Asiatic and a cultivated American species is between the chromosomes of the species involved in the hybrids rather than between chromosomes within the cultivated American species. Before Skovsted's suggestion can be accepted, it remains to be proved that the chromosomes of the Asiatic and the wild American species are nonhomologous.

Davie believes that the occurrence of several well-isolated wild American species characterized by 13 pairs of chromosomes indicates that the American species with 26 pairs probably arose through chromosome doubling in crosses between different wild American species. It was shown in the preceding paragraph that the chromosomes of *Gossypium harknessii* and *G. armourianum* are homologous to 13 of those of the cultivated American species. That this homology is between the same set of chromosomes in either *G. hirsutum* or *G. barbadense* is indicated by the fact that complete chromosome pairing occurs in F_1 of both *G. hirsutum* × *barbadense* and *G. harknessii* × *armourianum*.

Among the wild American species that may be involved in the origin of cultivated American cottons is *Thurberia thespesioides*. The writer finds that the somatic chromosomes of this species are slightly larger than those of the other wild American species. Skovsted (*24*) reports that the chromosomes of the cultivated American cottons may be equally divided into a larger and a smaller group. The differences of chromosome dimensions in cotton are very slight, however, and the writer's observations indicate a gradation in size of the chromosomes of the cultivated American species rather than a sharp segregation of large and small ones.

In the cultivated American species Webber (*27*) found that during meiosis quadrivalent chromosomes are occasionally formed. If the assumption that these species were derived from a cross between two modified tetraploids is correct, then such quadrivalent formation is easily explained upon the basis of chromosome homologies known to exist in similar modified octoploids. Such an explanation would also account for the occasional formation of quadrivalents in (1) hybrids between the species of the cultivated American group of cottons, (2) hybrids between the latter species and the wild American species, and (3) the natural hybrid between an Asiatic and a cultivated American species.

In the natural hybrid between an Asiatic and a cultivated American species, the composition of the pollen mother cells exhibiting quadrivalent chromosomes was found to be $1_{IV}+11_{II}+13_I$, $2_{IV}+9_{II}+13_I$, $3_{IV}+7_{II}+13_I$, or $4_{IV}+6_{II}+11_I$. Except in the last class, the quadrivalent group is followed by a decrease of 2 in the bivalent group, and in these three classes the number of univalents remains constant. Hence, it seems logical to assume that the quadrivalents are composed of chromosomes that generally form bivalents. If such an assumption is correct, then one of the bivalent chromosomes in the exceptional class just mentioned must be composed of chromosomes that generally form univalents.

SUMMARY AND CONCLUSIONS

In *Gossypium*, cross-pollinations made in the morning are more successful than those made in the afternoon. Those made in the late summer are more successful than those made in early summer. By removing the entire corolla and androecium during emasculation, rather than the anthers only, the percentage of successful cross-pollinations is greatly increased.

The correlation of the degree of compatibility between different species with their morphological similarity is often very limited. It is often less difficult to obtain hybrids between species of different taxonomic groups than between species of the same taxonomic group.

Hybrids between and within the following five morphologically distinct groups of *Gossypium* are described: (1) Cultivated American species ($n=26$); (2) wild American species ($n=13$); (3) cultivated Asiatic species ($n=13$); (4) a wild Australian species, *G. sturtii* ($n=13$); and (5) *Thurberia thespesioides* ($n=13$) a wild American plant, possibly congeneric with *Gossypium*.

Usually, in interspecific *Gossypium* hybrids the morphological similarity of the parental species and the normality of the heterotypic divisions are correlated. Also, the degree of fertility is correlated with the amount of chromosome pairing.

Certain characters show a tendency to dominance in interspecific hybrids of *Gossypium*. Other characters are expressed in a more or less intermediate degree.

Interspecific hybrids within the same group exhibit normal meiotic behavior. Hybrids between species having 26 pairs of chromosomes, like their parents, occasionally form quadrivalent chromosomes. Although hybrids between cultivated American and wild American species generally form 13 bivalent and 13 univalent chromosomes, they also occasionally form quadrivalent chromosomes. The latter behavior is also characteristic of a natural hybrid between an Asiatic and a cultivated American cotton. *Gossypium barbadense* × *sturtii* usually exhibits no chromosome pairing, but occasionally as many as 4 bivalent chromosomes have been observed. Hybrids between wild American species and *G. sturtii* likewise exhibit variable pairing. In the latter case, however, pairing is more frequent. Hybrids between *Thurberia thespesioides* and *G. sturtii* exhibit no chromosome pairing.

The occurrence of limited chromosome pairing in hybrids between wild American species and *Gossypium sturtii* and the formation of quadrivalents in hybrids between cultivated American and wild American species, and in a natural hybrid between an Asiatic and a cultivated American cotton, seem to support the hypothesis that the species having 13 pairs of chromosomes are modified tetraploids. If such is the case, then the lack of pairing in *Thurberia thespesioides* × *G. sturtii* must indicate that the chromosomes within the haploid sets of these two species have differentiated genetically to such an extent that pairing within either haploid set is impossible.

The formation of 13 bivalent chromosomes in hybrids between cultivated American and wild American species and in a natural hybrid between an Asiatic and a cultivated American cotton and the very limited pairing in *Gossypium barbadense* × *sturtii* support the hypothesis that the species having 26 pairs are allotetraploids. Such

an origin possibly involved species or close allies of some two of the following groups: Wild American species of *Gossypium*, *Thurberia hespesioides*, and Asiatic species of *Gossypium*.

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OCCURRENCE OF SELENIUM IN NATURAL PHOSPHATES, SUPERPHOSPHATES, AND PHOSPHORIC ACID [1]

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INTRODUCTION

The occurrence, origin, and distribution of selenium in soils, its absorption by plants, and the toxicity of the plants to animals have been discussed in several recent papers (*3*, *7*, *8*, *9*, *13*, *18*).[3]

With regard to the limits in the quantities of selenium that may be present in soil and vegetation without injury to animals, Byers (*3*, *p. 44*) states:

> In general it would appear that any soil containing upwards of 0.5 p. p. m. of selenium, and any vegetation containing 5 p. p. m. is potentially dangerous.

More than a decade ago Stoklasa (*22*) pointed out that the selenium content of soil may be greatly increased through heavy fertilization with superphosphate and ammonium sulphate. That the quantities of selenium occurring in fertilizers and soil amendments is sufficient to alter the amount the plant would otherwise absorb from the soil appears possible, when due consideration is given to the fact that these agents are applied to the layers of soil most readily accessible to the roots of the plant during its growing period, and often, particularly in the case of fertilizers, in relatively large quantities very near the root system. For example, let it be supposed that a fertilizer containing 50 p. p. m. of selenium is applied to the soil at the rate of 1,000 pounds per acre within 3 inches of the plant rows which are 3 feet apart. Then, assuming the customary figure, 2,000,000 pounds of topsoil to a depth of 6 inches per acre, the increase in the selenium concentration of the entire soil layer would be 0.025 p. p. m., and the increase in the concentration in a 6-inch layer of topsoil extending 3 inches on either side of the row, if all the selenium were retained therein, would be about 0.15 p. p. m. Under certain conditions the latter figure may represent a significant increase in the selenium content of the soil.

Results are given in this paper for selenium in 96 representative samples of phosphate rock and 3 samples of apatite from various deposits of the world, 8 typical samples of domestic superphosphate, and 4 samples of crude phosphoric acid manufactured by the sulphuric acid process. The results for selenium in natural phosphates reported herein represent an extension of the studies of this Bureau on the composition of phosphate rock (*11*, *16*).

PROCEDURE AND REAGENTS USED

The procedure used in the determination of selenium was substantially the same as that developed by Robinson, Dudley, Williams, and Byers (*19*) for selenium in soil and in sulphide-bearing rocks

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[2] The authors are indebted to K. D. Jacob, who suggested the investigation and rendered valuable aid during the course of the work, and to H. G. Byers and K. T. Williams, Division of Soil Chemistry and Physics, Bureau of Chemistry and Soils, for helpful suggestions in the application of the analytical methods.

[3] Reference is made by number (italic) to Literature Cited, p. 1082.

and minerals, according to which the selenium is separated and concentrated by distillation with hydrobromic acid in the presence of bromine and is determined in the distillate by gravimetric methods, or by a colorimetric procedure when the absolute amount of selenium is less that 0.5 mg. Three distillation procedures are described for inorganic materials: (1) Direct distillation of the sample, applicable to soil and nonpyritiferous rocks and minerals; (2) distillation after the sample has been pretreated with nitric acid to destroy sulphides, and finally with concentrated sulphuric acid to eliminate the nitrate radical which otherwise would decompose the hydrobromic acid in the subsequent distillation, applicable to materials containing pyrite and other sulphides; and (3) integrated distillation, a method of concentration in which the distillate from one portion of the sample is added in the distillation of a fresh portion and so on, which is especially adapted to the determination of traces of selenium.

Phosphate rock presents some difficulties in the application of these methods. As a result of the presence of considerable quantities of fluorine and silica, relatively large quantities of gelatinous silica are rapidly deposited in the condenser during the early stages of distillation, and when more than about 10 g of the samples are used, the condenser often becomes clogged. Furthermore, the amount of gelatinous silica that separates in the distillate when large samples are used is sufficient seriously to retard subsequent filtration. These difficulties are intensified in the integrated distillation. For a calcium-rich material like phosphate rock, sulphuric acid is not satisfactory for the expulsion of the nitrate radical following the preliminary treatment of pyritiferous samples, because the relatively insoluble calcium sulphate causes serious bumping during subsequent distillation. Phosphoric acid is recommended for this purpose.

Selenium can be removed from dry soil by distillation with hydrobromic acid with a specific gravity as low as 1.2.[4] Accordingly, acid sufficiently concentrated for use in the distillation can be readily recovered by redistilling the filtrates and solutions from the estimation of the selenium. The specific gravity of the recovered acid used by the writers ranged between 1.35 and 1.40. When 10 g of 100-mesh pyrite-free rock was distilled with 100 ml of acid of this strength, all the selenium passed into the first 50 ml of the distillate. In samples that carried only small amounts of acid-insoluble sulphides, selenium was detected in the second 50 ml of the distillate but not thereafter. In view of these findings the volume of distillate collected was 75 to 150 ml, depending on the character of the sample. It should be mentioned that the difficulties with the separation of gelatinous silica are less marked when the more dilute acid is used.

Bromine often contains impurities capable of imparting to the distillate a color which, since it persists after the solution is reduced with sulphur dioxide, interferes in the colorimetric comparison. The interfering substance can be eliminated by dissolving the bromine in hydrobromic acid, filtering off the flocculent precipitate,[5] and distilling the filtered solution.

[4] Byers, H. G. Private communication.

[5] The nearly white, waxy substance (0.232 g of air-dried material) obtained by adding 30 ml of bromine to 600 ml of water-white hydrobromic acid (48 percent), allowing the mixture to stand an hour, filtering, and washing the precipitate with cold water, was soluble in alcohol and ether. Somewhat more than one-half of the substance melted at 136° to 140° C.; the remainder did not melt at 160° C. When it was subjected to steam distillation about two-thirds of the wax passed over with the steam.

In order to compare the performance of the three distillation methods mentioned, these procedures were used to determine the selenium in several typical phosphate rocks. The results (table 1) indicate that, even though the rock contains relatively large quantities of organic matter, pretreatment of the sample is not necessary, unless acid-insoluble sulphides are present and then only when the highest accuracy is desired. Unless it is stated otherwise, the results for selenium reported in this paper were obtained by distilling the samples without pretreatment.

TABLE 1.—*Selenium in typical phosphate rocks as determined by three different distillation procedures*

Sample no.	Type or source of phosphate	FeS_2	Organic carbon	Selenium determined by—		
				Distilling 10 g of sample		Integrated distillation of untreated sample[1]
				Untreated	Pretreated	
		Percent	*Percent*	*P. p. m.*	*P. p. m.*	*P. p. m.*
973	Idaho	0.00	2.34	28	28	(2)
948	Wyoming	.97	3.47	48	48	(2)
449	Tennessee blue rock	2.39	(2)	≦.8	1.0	(2)
930	——do	3.90	.20	2.0	3.0	(2)
1139	South Carolina land rock	.36	(2)	14	16	(2)
912	Florida land pebble	.00	.38	≦.8	(2)	0.8
908	Tennessee brown rock	.00	.11	≦.8	(2)	<.1
916	Tennessee phosphatic limestone	.73	(2)	≦.8	(2)	<.1
917	——do	1.89	(2)	≦.8	(2)	.2

[1] 100 g of the sample was distilled in 10-g portions.
[2] Not determined.

Under the conditions prescribed (*19*) for the colorimetric estimation, the threshold sensitivity of the method is about 0.008 mg of selenium. For 10 g of sample this figure corresponds to 0.8 p. p. m., and accordingly the results are reported as $\leqq$0.8 p. p. m. in those cases where the coloration was barely perceptible and as $<$0.8 where no color was noted. When 100 g of the sample are used (integrated distillation) the detectable concentration of selenium is thereby reduced to approximately 0.1 p. p. m.

SELENIUM IN NATURAL PHOSPHATES

Results for selenium in domestic and foreign phosphates are given in tables 2 and 3, respectively. All the samples from Florida, Tennessee (except Tennessee blue rock), Kentucky, Arkansas, Oklahoma, and Australia, the light-colored phosphates from the western part of the United States, and the apatites from Virginia and Canada contained 1 p. p. m. or less of selenium. The results for the dark-colored phosphates of the western part of the United States and Canada, the majority of the Tennessee blue-rock and South Carolina samples, and the African and Palestinian phosphates ranged between 1 and 55 p. p. m. A few of the samples from European and insular deposits contained as much as 1 to 2 p. p. m. of selenium.

TABLE 2.—*Selenium content of domestic phosphates*

Sample no.	Type of phosphate	Location of deposit	P_2O_5	Se
			Percent	*P. p. m.*
FLORIDA PHOSPHATES				
910	Land pebble	Mulberry	31.09	<0.8
947	do	Brewster	31.28	<.8
790	do	Not known	31.40	<.8
912	do	Mulberry	35.37	[1].8
771	Hard rock	Not known	31.25	<.8
589	do	Floral City	34.68	<.8
932	do	Dunnellon	35.99	<.8
1091	Soft	Bartow	25.47	<.8
728	do	Juliette	31.80	<.8
915	Waste pond	Dunnellon	23.63	<.8
SOUTH CAROLINA PHOSPHATES				
495	Not known	Not known	16.07	<0.8
1139	Land rock	Bulow mines, Johns Island	26.92	[2]16.0
1138	do	Lamb's mine, near Charleston	27.85	8.5
650	do	Not known	28.86	5.0
TENNESSEE PHOSPHATES				
[3]56	Brown rock	Not known	31.28	<0.8
906	do	Wales	34.39	<.8
908	do	Mountpleasant	34.44	[1]<.1
772	Blue rock	Glover	30.45	<.8
930	do	Gordonsburg	30.97	[2]3.0
448	do	Glover	32.03	2.5
449	do	Gordonsburg	32.03	[2]1.0
1049	Kidney phosphate	Boma	31.22	<.8
1048	White rock	Tomscreek	30.20	<.8
1031	do	Godwin	35.80	<.8
916	Phosphatic limestone	Mountpleasant	11.22	[1]<.1
917	do	Gordonsburg	11.68	[1].2
WESTERN PHOSPHATES				
550	Light colored	Idaho, Paris	32.21	<0.8
1412	do	do	35.39	1.0
1011	do	Montana, Garrison	27.63	<.8
1018	do	do	29.11	<.8
1019	do	do	31.47	<.8
1017	do	do	34.92	<.8
1012	do	do	36.07	<.8
1252	do	do	36.38	<.8
1010	do	do	37.47	1.0
1407	do	do	37.93	<.8
1411	Dark colored	Idaho, Georgetown	30.29	50
489	do	do	31.97	6.0
973	do	Idaho, Conda	31.97	[2]28
1253	do	do	32.13	8.0
454	do	do	32.24	40
1408	do	do	32.26	23
494	do	Idaho, Georgetown	34.96	8.0
1280	do	Montana, Maxville	24.95	6.0
1410	do	Utah, Devils Slide	11.90	38
1409	do	Utah, Logan	31.50	10
467	do	Wyoming, Cokeville	26.60	35
469	do	do	29.75	50
468	do	do	29.79	55
948	do	do	30.19	[3]48
OTHER PHOSPHATES				
1267		Arkansas, Independence County	31.98	<0.8
1235	Brown rock	Kentucky, Wallace	21.19	<.8
1242		Oklahoma, Cotton County	24.31	<.8
1295	Apatite	Virginia, Amherst County	39.58	<.8

[1] Result obtained by integrated distillation.
[2] Result obtained by distillation of pretreated sample.
[3] National Bureau of Standards sample no. 56.

TABLE 3.—*Selenium content of foreign phosphates*

AFRICAN PHOSPHATES

Sample no.	Location of deposit	P_2O_5	Se	Sample no.	Location of deposit	P_2O_5	Se
		Per cent	*P. p. m.*			*Per cent*	*P. p. m.*
560	Algeria, Dyr	23.39	18.0	453	Morocco	33.47	5.0
551	Algeria, Tebessa	26.10	2.5	563	do	34.30	1.5
558	Algeria, Rebiba	26.84	3.0	1162	do	35.11	1.0
562	Algeria, M'Zaita	28.59	7.5	552	Tunis, Gafsa	26.91	18.0
557	Algeria, Tocqueville	29.38	18.0	556	Tunis, Kalaa-Djerda	27.55	30.0
559	Algeria, Bordj-Redir	32.34	55.0	561	Tunis, M'Dilla	28.66	3.5
555	Egypt, Kosseir	30.60	1.0	553	Tunis, Gafsa	29.13	1.0

EUROPEAN AND ASIAN PHOSPHATES

1226	Belgium, Liége	18.13	<0.8	1263	U. S. S. R., Saratov	18.40	<0.8
1155	Estonia, Tallinn	25.68	<.8	1266	U. S. S. R., Egoriev	19.50	1.5
1228	France, Somme	22.02	1.5	1264	do	22.37	1.0
1240	do	24.66	<.8	1262	U. S. S. R., Vyatka	27.88	<.8
1241	France, Pyrenees Mountains	26.87	2.0	[1]1260	U. S. S. R., Kola Peninsula	39.08	<.8
1239	France, Quercy	34.74	1.0	1258	Palestine, Neby Musa	8.14	1.5
1151	Portugal, Marvão	27.14	<.8	1255	do	16.60	1.0
1152	U. S. S. R., Volga River region	13.40	<.8	1256	do	17.50	1.5
1265	U. S. S. R., Aktyubinsk, Siberia	17.42	<.8	1257	do	20.40	15.0

INSULAR PHOSPHATES

1223	Angaur Island	40.00	<0.8	943	Curaçao Island	40.66	1.0
452	Christmas Island	39.46	<.8	1159	Makatea Island	38.22	<.8
904	Grand Connetable Island	54.51	2.0	450	Nauru Island	38.92	<.8
985	Curaçao Island	38.59	1.5	451	Ocean Island	40.32	<.8

OTHER PHOSPHATES

1157	South Australia, Kapunda	30.18	<0.8	[2]582	Canada, British Columbia	24.11	3.0
1158	South Australia, Port Clinton	33.53	<.8	[1]905	Canada, Quebec Province	40.30	<.8

[1] Apatite.
[2] A dark-colored phosphate from Crow's Nest Pass.

SELENIUM-BEARING CONSTITUENTS

In general, selenium is found in nature associated with sulphur, particularly the sulphides (*4*, *5*, *17*) and in a number of rare selenide minerals analagous to the sulphides (*17*, *pp. 693–695*). Byers (*3*) concluded that the selenium in soils came from sulphide minerals in the soil parent materials. Since selenium under certain conditions is absorbed by growing plants and is associated with the protein (*18*), organic matter originating from vegetation may be regarded as a potential source of selenium in sedimentary rocks. If the organic matter represents the remains of plant life that grew on seleniferous soil under climatic conditions favorable to selenium absorption, it may easily be the principal carrier of selenium.

In view of the foregoing some relationship may be expected to exist between the selenium content of phosphate rock and the quantities of sulphide sulphur, organic carbon, and nitrogen present. According to the comparative data (table 4), larger amounts of selenium occur in pyritiferous than in pyrite-free samples from the same region, and as a rule rocks containing the greater amounts of organic matter also carry the larger quantities of selenium. The latter relationship is best shown by the Permian phosphates of the western part of the

United States, and at least in the case of these phosphates it may be regarded as evidence that the organic matter is seleniferous. The nitrogen figures show somewhat less correlation with the selenium results than do the figures for organic carbon. The sample of Tennessee kidney phosphate is a notable exception to these general relationships.

TABLE 4.—*Comparative results for selenium, organic carbon, nitrogen, and pyritic sulphur in phosphate rock*

Source or type of phosphate	Organic carbon	Se	N	FeS_2
Western part of the United States:	*Percent*	*P. p. m.*	*P. p. m.*	*Percent*
Dark-colored pyritiferous rock	[1] 3.90	[1] 30.0	1,100	[2] 1.92
Dark-colored pyrite-free rock	[1] 2.60	[1] 13.0	[2] 1,000	.0
Light-colored rock	[1] .16	[1] < .9	80	.0
Tennessee kidney phosphate	1.46	< .8	2,600	1.5
Tunis, Gafsa	.86	18.0	470	.0
South Carolina	.51	8.5	510	.3
Florida [1]	.31	< .8	170	.0
Grand Connetable Island	.28	2.0	490	.0
Tennessee blue rock	.28	< 1.4	280	3.0
Curaçao Island	.17	1.5	130	.0
Morocco	.17	1.0	180	.0
Tennessee brown rock and Tennessee white rock	.16	< .8	160	[3]

[1] Average of results for 3 or more samples.
[2] Average of results for 2 samples.
[3] The 1 pyritiferous brown rock, National Bureau of Standards standard sample no. 56, contained sulphide equivalent to 0.89 percent of FeS_2.

Results for organic carbon and selenium in mechanical separates of ground phosphate rock are given in table 5. The correlation between the results for selenium and organic carbon is probably as close as could be expected, particularly in the sample of Wyoming phosphate. In the Idaho sample the results indicate considerable loss of selenium occasioned by suspension of the material in water incident to mechanical separation into fractions.

TABLE 5.—*Distribution of selenium among mechanical separates of ground phosphate rock*

Mechanical fraction [1]	Idaho phosphate no. 973			Wyoming phosphate no. 948			
	Fraction of original material	Organic carbon	Se	Fraction of original material	Organic carbon	Se	FeS_2
	Percent	*Percent*	*P. p. m.*	*Percent*	*Percent*	*P. p. m.*	*Percent*
"Sand"	26.0	2.22	33	49.8	2.96	36	0.62
"Silt"	45.5	2.03	20	43.6	4.25	56	1.17
"Clay"	27.9	3.03	18	6.6	8.29	64	.42
Solution loss	.6	----------	[2] 5	.0	----------	[2] 2	----------
Original material	100.0	2.34	28	100.0	3.47	48	.97

[1] The mechanical fractions of these phosphates were prepared by Alexander and Jacob (*1*) from material ground to pass a 100-mesh sieve.
[2] Calculated.

PRIMARY AND SECONDARY DEPOSITS

Following Blackwelder's division (*2*) of the world's phosphate deposits into six genetic varieties, comprised in two broad groups (primary and secondary deposits), Mansfield (*15, p. 362*) has partially

classified a number of the deposits. Accordingly, as far as possible the results for selenium in phosphate are summarized under the two general groups in table 6.

TABLE 6.—*Selenium content of phosphate rocks from primary and secondary deposits*

Type of deposits and location	Samples analyzed	Selenium	
		Range	Average
Primary:	*Number*	*P. p. m.*	*P. p. m.*
Western part of the United States and Canada	25	<0.8–55	<16.7
Algeria, Tunis, and Egypt	11	1.0–55	14.3
Tennessee (blue rock) and Arkansas	5	<.8–3.0	<1.6
Average			<10.9
Secondary:			
South Carolina	4	<.8–16	<7.6
France	4	<.8–2.0	<1.3
Islands	8	<.8–2.0	<1.1
Florida	10	<.8	<.8
Tennessee and Kentucky (brown rock and white rock)	6	<.8	<.6
South Australia	2	<.8	<.8
Average			<2.0

Byers' conclusion (*3*) that selenium is leached from soil by percolating waters, which is supported by the presence of considerable amounts of this element in drainage water (table 8), and the fact that a considerable part of the selenium in one phosphate rock (table 5) was removed when the material was treated with water incident to mechanical analysis, afford good reasons to expect primary deposits, having been protected from the action of water, to carry larger quantities of selenium than secondary deposits. Considering the average figures for the groups (table 6), the primary deposits show about five times as much selenium as the secondary deposits, and the expectation is, in general, thus confirmed. It should be pointed out, however, that this criterion fails when it is applied to certain individual deposits. For example, South Carolina phosphate, a secondary deposit, carries about fivefold as much selenium as does Tennessee blue rock, a primary deposit. In this connection special interest attaches to the experimental finding (*9*) that the absorption and retention of added selenium is much more pronounced in some soils than in others, and to the suggestion that this difference in the behavior of the selenium might be due to the difference in the composition of the soil colloids.

GEOLOGIC AGE AND SELENIUM CONTENT

In table 7 the results for selenium in natural phosphates are grouped, as far as possible, according to the geologic age of the deposits (*10*). Of the deposits for which the average result is greater than 1 p. p. m., the Permian phosphates show the largest amount of selenium and the post-Tertiary the least. In the descending order of selenium content, the Miocene, Cretaceous (or Jurassic), Eocene, and Devonian phosphates are between these extremes.

TABLE 7.—*Geologic age as correlated with selenium content of natural phosphates*

Period or epoch	Source or type of phosphate	Samples analyzed	Selenium	
			Range	Average
		Number	*P. p. m.*	*P. p. m.*
Post-Tertiary	Island phosphates	8	<0.8–2.0	<1.1
Tertiary	Florida, South Carolina, Morocco, Australia, and Russia.	20	<.8–16	<2.4
Pliocene	Florida land pebble	4	≦.8	<.8
Miocene	South Carolina land rock	3	5.0–16	9.8
Oligocene	Florida hard rock and soft phosphate	6	≦.8	<.8
Eocene	Morocco	3	1.0–5.0	2.5
Cretaceous (or Jurassic)	France, Russia, Algeria, Tunis, and Egypt.	19	<.8–55	<8.8
Carboniferous	Western United States and Canada	25	<.8–55	<16.7
Permian	Dark-colored phosphates [1]	15	3.0–55	27
Do	Light-colored phosphates [2]	10	<.8–1.0	<.8
Mississippian	Tennessee kidney phosphate	1	≦.8	≦.8
Devonian	Tennessee blue rock	4	≦.8–31	≦1.8
Ordovician	Tennessee and Kentucky brown rock [3]	8	<.8	<.6
Pre-Cambrian	Apatite from Virginia and Canada	2	<.8	<.8

[1] From Canada; Georgetown, Idaho; Maxville, Mont.; Cokeville, Wyo.; and British Columbia.
[2] From Paris, Idaho; and Garrison, Mont.
[3] Also 2 samples of Tennessee phosphatic limestone, 1 sample of Arkansas phosphate, and 1 sample of Estonian phosphate.

In view of previous discussion, it is hardly necessary to remark that other factors may play a greater part than geologic age in limiting the amounts of selenium that occur in any given deposit. As to the geologic age, a comparison of primary deposits is most logical. Accordingly, the geologic periods during which the primary phosphate deposits were laid down are, in the increasing order of average selenium content of the phosphates, Devonian, Cretaceous, and Permian.

PHOSPHATES COMPARED WITH OTHER ROCKS, MINERALS, SOIL, AND WATER

The available results for selenium in rocks, minerals, soils, and waters of the United States are summarized in table 8. The analyses of all the materials other than phosphates were recently published by Byers (*3*) and Williams and Byers (*25*). Since the data are by far the most extensive for certain localities west of the Mississippi River, mainly parts of Colorado, Montana, Nebraska, South Dakota, and Wyoming, the results have been classified under two geographic divisions of the country, the Mississippi River being used as the dividing line. The results of Goldschmidt and Hefter (*4*) and Goldschmidt and Strock (*5*) for selenium in certain genetic varieties of rocks and minerals, particularly sulphides, are summarized in table 9, in which the results for phosphates (table 6) are also included for convenience in comparison.

According to the available data (table 8), phosphate rock from deposits east of the Mississippi River contains less selenium than does that from deposits in the West. Aside from a crude sulphur from Colorado showing 8,350 p. p. m., pyrite, as would be expected, contained the largest quantities of selenium. The selenium content of phosphate rock from the western division of the United States agrees very well with that of other sedimentary deposits in this region. In the ascending order of the average results, shale, chalk, Permian phosphate, and limestone lie between the values (9 and 20 p. p. m., respectively) for ironstone (and mudstone) and bentonite.

TABLE 8.—*Selenium in rocks, minerals, soils, and waters of the United States west and east of the Mississippi River*

Location and material	Samples analyzed	Selenium	
		Range	Average
	Number	*P. p. m.*	*P. p. m.*
West of Mississippi River:			
Phosphate rock (Permian only)	24	<0.8–55	<17.2
Limestone	6	1.5–46	19
Sandstone	1	----------	.4
Chalk (Niobrara)	17	6.0–30	15
Gypsum	4	1.0–10	4.6
Gypsum sand	1	----------	.2
Sulphur (crude from Colorado)	1	----------	8,350
Pyrite	6	5.0–320	123
Ironstone and mudstone	4	4.0–14	9.0
Shale	80	.0–103	11
Bentonite	6	2.0–76	20
Soil	446	.0–41	2.0
Well water	12	.02–.2	.06
Creek and drainage water	6	.00–1.2	.2
East of Mississippi River:			
Phosphate rock	25	<.8–16	<2.7
Apatite	1	----------	<.8
Pyrite	20	.0–230	70
Marcasite (from clay)	2	.3–.6	4.5
Pyrrhotite	1	----------	5.0
Chalcopyrite	1	----------	10
Mispickel	1	----------	(1)
Shale	5	.15–.6	.25
Clay bed	[2] 6	.0–.4	.18

[1] Trace.
[2] Samples of surface soil, sand, and clay.

TABLE 9.—*Selenium in certain genetic varieties of minerals and rocks, nitrate beds, and waters*

Material	Samples analyzed	Selenium	
		Range	Average
	Number	*P. p. m.*	*P. p. m.*
Meteorites (iron, troilite, and chondite)	5	1.5–132	47
Sulphides:			
Primary magmatic [1]	7	17–65	44
Pneumatolytic and hydrothermal [2]	12	.8–190	29
From sedimentary deposits [3]	13	.1–32	12
Oxides of iron and manganese [4]	5	.5–5.4	2.0
Phosphates: [5]			
Primary deposits	41	<.8–55	<10.9
Secondary deposits	34	<.8–16	<2.0
Limestone, Devonian	(6)	----------	.1
Argillaceous-arenaceous rocks	(7)	----------	.6
Chilean nitrate beds, caliche	(8)	----------	5.2
Water from North Sea	2	.0033–.0044	.0038
Water from La Roche-Posay	3	----------	[9] .2

[1] Pyrrhotite from Germany, Norway, South Africa, and Ontario, Canada.
[2] From Norway, Germany, England, California, and Colorado.
[3] 1 sample from Newfoundland, others from Germany.
[4] 1 sample was a deep-sea nodule of manganese ore, others from Germany, Finland, and Newfoundland.
[5] Results from table 6.
[6] Composite sample representative of 32 limestones of Germany.
[7] Composite sample representative of deposits in Germany and Japan.
[8] Composite sample representative of 14 localities.
[9] Analyses by Taboury (*23*).

When all the data are considered, it appears that sedimentary deposits, in general, carry less selenium than other deposits. Furthermore, larger quantities of selenium occur in primary than in secondary deposits.

SELENIUM IN SUPERPHOSPHATE AND PHOSPHORIC ACID

In view of the occurrence of selenium in phosphate rock in amounts ranging from <0.1 to 55 p. p. m. (tables 2 and 3), notable quantities of this element would ordinarily be expected in superphosphate and phosphoric acid manufactured from rock from certain phosphate deposits. Furthermore, selenium may be introduced into superphosphate and phosphoric acid as an impurity in the sulphuric acid used to decompose the rock. Considerable quantities of selenium are often present in sulphuric acid manufactured from pyrite or produced as a byproduct of the smelting of other sulphide ores; acid from these sources is used extensively in the fertilizer industry, particularly in the manufacture of superphosphate. Stoklasa (*22*) reported 26 to 58 p. p. m. of selenium in Glover tower acid and 15 to 42 p. p. m. in chamber acid. A sample of byproduct sulphuric acid (60° B.) from a copper-smelting operation in Tennessee contained 52 p. p. m. of selenium (*6*). The presence of selenium in sulphuric acid has also been noted by others (*20*, *24*). Inasmuch as selenium occurs in coal (*12*) and also in coke (*21*), phosphoric acid produced by the electric-furnace and blast-furnace processes, particularly the latter, may also carry selenium as the result of using coke as a reducing agent and as fuel.

Stoklasa (*22*) found 15 to 36 p. p. m. of selenium in European superphosphate. Much smaller quantities were found in American superphosphates by the authors. The results (table 10) range from <0.8 to 4.0 p. p. m. As the samples were typical commercial superphosphates recently made from phosphate rock representing the principal deposits in this country, it appears that these results may be regarded as a fairly accurate range for the selenium content of superphosphate manufactured in the United States at the present time.

TABLE 10.—*Selenium in superphosphate and phosphoric acid*

Sample no.	Type or source of phosphate rock	Source of sulphuric acid	P_2O_5	Se
		ORDINARY SUPERPHOSPHATE		
			Percent	*P. p. m.*
1315	Florida land pebble	Pyrite	19.20	1.5
1370	do	do	21.06	.8
1316	Tennessee brown rock	Sulphur	18.86	<.8
1414	Florida land pebble	Sludge acid from petroleum refining	17.85	<.8
1402	do	do	20.60	1.0
		DOUBLE SUPERPHOSPHATE		
1337	Florida land pebble	Sulphur	46.21	<0.8
1362	Tennessee brown rock	[1]	48.37	1.5
1372	Idaho	Copper-smelting operation in Montana	47.33	4.0
		CRUDE PHOSPHORIC ACID		
1200	Idaho	Copper-smelting operation in Montana	20.26	<0.07
1199	do	do	37.80	<.07
1058	Tennessee brown rock	Copper-smelting operation in Tennessee [2]	16.04	.14
1057	do	do	41.38	.5

[1] Phosphoric acid manufactured by the blast-furnace process was used.
[2] A sample of acid (60° B.) from this smelting operation contained 52 p. p. m. of selenium (*6*).

The results in table 10 present a few additional points of special interest. Only a very small part of the selenium in the raw materials was found in the superphosphate or phosphoric acid. For example, the phosphoric acids produced from Idaho phosphate by the sulphuric-acid process carried less than 0.07 p. p. m. of selenium, despite the fact that the rock (Conda, Idaho) contains 8 to 40 p. p. m. (table 2) and the copper ore from which the sulphuric acid was produced as a by-product was probably also seleniferous. On processing phosphate rock from Conda, Idaho, it is the practice to calcine the rock at about 700° C. (*14*), in order to eliminate most of the organic matter which otherwise would seriously foul the acid and apparatus. At least a portion of the selenium of the phosphate rock is probably volatilized by this treatment.[6] Doubtless a part of any selenium that is present in the mixture of rock and sulphuric acid remains with the sludge and is thus eliminated from the phosphoric acid. Furthermore, selenium may be lost by volatilization during concentration operations, although such a loss is not indicated by the results for the Tennessee acids. It may also be noted in this connection that, whereas a sample of sulphuric acid from the same source as that used in the manufacture of the Tennessee brown-rock acids carried 52 p. p. m. of selenium, the concentrated phosphoric acid contained only 0.5 p. p. m. and the dilute acid considerably less. Therefore, it would appear that, under the conditions of manufacture in this country, only a small part of the selenium occurring in the raw materials, in general, finds its way into the finished phosphate products.

SUMMARY

Results are given for selenium in 96 samples of phosphate rock and 3 samples of apatite from various deposits of the world, 8 representative samples of commercial superphosphates manufactured from domestic rock, and 4 samples of crude phosphoric acid produced by the sulphuric-acid process.

The results for selenium in natural phosphates range from <0.1 p. p. m. in a Tennessee brown rock to 55 p. p. m. in Wyoming and Algerian phosphates. The occurrence of selenium in natural phosphates is discussed from the following points of view: (1) Selenium-bearing constituents; (2) primary and secondary deposits; (3) geologic age of deposits; and (4) a comparison of phosphates with other geologic formations. Accordingly, the data indicate (1) that organic matter and, to a less extent, inorganic sulphides are important carriers of selenium in phosphate rock, (2) that primary deposits are in general richer in this element than are secondary deposits, (3) that deposits belonging to the Permian and Cretaceous ages contain the most selenium, and (4) that the selenium content of phosphate deposits is about the same as that of other sedimentary deposits in the same region.

The quantity of selenium in superphosphate ranges from <0.8 to 4.0 p. p. m., and in phosphoric acid is 0.5 p. p. m. or less. According to the available data only a small fraction of the selenium occurring in the natural materials from which superphosphate and phosphoric acid are made finds its way into the finished product.

[6] According to the results of a single experiment with Wyoming rock no. 948, a highly seleniferous phosphate (table 2), selenium was completely volatilized when the phosphate was calcined in the presence of water vapor at 1,400° C. for 30 minutes.

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ROOT SYSTEMS OF CERTAIN TREES AND SHRUBS GROWN ON PRAIRIE SOILS[1]

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INTRODUCTION

Actual data on the depth and spread of tree roots are not abundant, and such as there are deal mainly with fruit trees grown under fairly humid conditions. The study here described was made to determine the depth and spread of certain forest trees and shrubs growing in North Dakota. The work was begun in the autumn of 1934.

REVIEW OF LITERATURE

Zon (*20*)[3] states that the water table under the forest is lower than that outside, for the forest through transpiration consumes more water than any other cover crop, and draws the water from a greater depth. Halden (*7*) says that in the sterile zone on the borders of forest stands on south and west exposures the soil is drier than either inside or outside of the groves, and that this dryness is more pronounced on sand than on clay.

Goff (*6*), Ballantyne (*1*), Oskamp and Batjer (*11*), Partridge and Veatch (*12*), Clark (*3*), Goff (*5*), Rogers (*13*), and Mason (*10*) found the greater part of the roots of fruit trees in the upper 3 feet of soil and the maximum depth of penetration 9 feet.

Holch (*8*) stated that the greatest depth to which the roots of young forest seedlings penetrated during the first year was 5.7 feet.

Laitakari (*9*), in a study of Scotch pine (*Pinus sylvestris*), found that root systems extended far beyond the spread of branches, with a maximum depth of 3 meters and that spruce had a still shallower root system. Cheyney (*2*) found that jack pine also had a shallow root system, and Woodroof and Woodroof (*19*) reported that roots of pecan in Georgia rarely reach a depth of more than 5 feet.

Weaver and Kramer (*18*), studying oak (*Quercus macrocarpa*) in Nebraska, found roots with 2 to 4 times the radial spread of the branches. Deep roots reached 14 feet.

The foregoing observations hardly seem to support the common belief expressed by Zon (*21*) and Shepard (*13*) that tree roots penetrate to great depths.

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[2] Indebtedness is acknowledged to the Federal Transient Bureau of Fargo for supplying transients to assist in the work, and to the transients themselves for their splendid cooperation; to Marvel Lien who supervised the work; and to Prof. C. B. Waldron who supplied the information on the ages of the plantations.

[3] Reference is made by number (italic) to Literature Cited, p. 1091.

LOCATION AND MATERIALS

Most of the studies here recorded were made on the grounds of the North Dakota Agricultural College and Agricultural Experiment Station at Fargo. The soil is a Fargo clay, such as characterizes parts of the Red River Valley. It is very level and is sometimes deficient in drainage during the early spring months. The black surface soil extends downward to a depth of about 3 feet, where it changes to a light-colored calcareous, clayey subsoil. There is no true hardpan and no stratum of rock. Because of the continued deficiency of rainfall for many years the soil has become very dry and the water table is nowhere higher than 15 feet.

To determine the behavior of tree roots under different soil conditions, a snow trap planted by the Northern Pacific Railway Co. along its right-of-way was also studied. This planting, 25 years old, is located 38 miles west of Fargo, just outside the Red River Valley, in Barnes loam, a light soil.

METHODS

In general, the following method was employed in making these studies: Where there was a row of trees, as there was in most cases, a trench 18 inches to 2 feet in width was dug parallel to the row and under the outer edge of the branches of the trees. The digging was deep enough to sever all roots. After the trench was dug the upper 1 foot was separated from the remainder by a cord stretched parallel to the ground surface, and the roots which came through the trench wall in this area were counted and classified according to diameter. After this was done, the second foot layer was handled in the same way, then the third, the fourth, and so on until a depth was reached where no more roots were found. Later the larger roots which crossed the trench were dug out. They were traced from the point where they left the trench until they became too small to follow.

In cases where it was thought that root penetration might be deeper beneath the tree than farther from it, excavations were made directly under the tree. When it was suspected that the first specimens might not represent a fair sample of the species, and other plants were available, these were studied also. By such means the depth and distribution of the root systems in the various soil levels were ascertained, and the maximum spread of the roots in the soil and their maximum depths were determined and tabulated. In addition, a limited number of soil-moisture determinations were made.

PRESENTATION OF DATA

ROOT DISTRIBUTION IN FARGO CLAY AND BARNES LOAM

Table 1 shows a compilation of data secured from root studies with 31 species of trees and shrubs growing on Fargo clay at Fargo, N. Dak., without irrigation. Trenches made in studying these species ranged from 20 feet to 600 feet in length for each species. According to the United States Weather Bureau records, the mean normal rainfall at Fargo is 22.34 inches per year, but for the past 15 years the average has been only 17.78 inches and for the past 6 years, 15.43 inches.

TABLE 1.—*Depth of penetration and lateral spread of tree and shrub roots in Fargo clay*

Name	Age	Height	Length of longest roots	Ratio of root length to height	Proportion of roots in—							Greatest depth of roots	Depth of largest roots
					First foot	Second foot	Third foot	Fourth foot	Fifth foot	Sixth foot	Seventh foot		
	Years	*Feet*	*Ft. In.*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Ft. In.*	*Ft. In.*
Acer ginnala, Amur maple	10	12	21 6	1.7	32.8	42.7	19.6	4.7	None	None	None	3 6	12 36
Acer negunda, box elder	40	35	46 0	1.3	11.3	41.3	31.6	15.5	None	None	None	4 2	12 24
Acer saccharinum, soft maple	35	45	49 0	1.0	21.4	40.6	24.2	13.6	None	None	None	4 3	6 24
Caragana arborescens, Siberian pea-tree	38	17	21 6	1.2	24.0	30.9	25.2	11.8	7.9	None	None	5 6	12 24
Celtis occidentalis, hackberry	35	35	41 6	1.2	31.8	43.7	15.2	5.7	3.4	None	None	4 5	4 12
Elaeagnus angustifolia, Russian olive	25	26	39 0	1.5	23.4	26.5	31.6	18.3	None	None	None	5 10	24 36
Fraxinus lanceolata, green ash	43	38	48 0	1.2	26.1	37.1	29.1	7.5	None	None	None	3 7	12 24
Juglans cinerea, butternut	10	10	16 10	1.6	65.4	26.3	8.2	None	None	None	None	2 8	4 12
Juglans nigra, black walnut	25	25	54 0	2.1	41.4	46.7	9.7	2.1	None	None	None	5 2	10 20
Juniperus scopulorum, Colorado juniper	25	20	23 0	1.1	36.3	48.8	13.0	1.8	None	None	None	3 3	12 24
Juniperus virginiana, red cedar	25	20	22 3	1.1	41.8	37.1	19.3	1.6	None	None	None	3 3	12 24
Lonicera tatarica, Tartarian honeysuckle	10	15	16 6	1.1	19.3	38.2	25.9	16.4	None	None	None	3 6	4 12
Malus baccata, Siberian crab apple	40	19	34 10	1.8	38.0	29.8	20.9	4.3	2.8	2.0	1.9	6 10	6 24
Malus sylvestris, apple (Hibernal)	16	16	26 0	1.6	38.7	29.6	25.5	6.0	None	None	None	10 3	6 36
Picea canadensis, Black Hills, form of white spruce	11	11	8 0	.7	25.0	33.8	29.4	11.7	None	None	None	3 6	24 36
Picea pungens, Colorado spruce	39	35	16 9	.4	40.8	32.9	17.5	8.5	None	None	None	3 5	12 24
Pinus banksiana, jack pine	32	20	38 6	1.9	32.7	41.0	17.8	8.3	None	None	None	3 5	6 24
Pinus ponderosa, western yellow pine	32	25	20 3	.8	35.2	36.9	21.3	6.4	None	None	None	3 8	12 24
Populus monilifera, northern cottonwood	43	65	75 0	1.1	32.0	35.1	20.4	10.6	1.7	None	None	10 0	12 36
Prunus americana, American plum	10	10	10 6	1.1	40.3	32.7	26.8	None	None	None	None	2 10	6 30
Prunus virginiana, chokecherry	12	16	35 0	2.1	27.8	36.8	17.6	10.0	7.6	None	None	6 4	6 24
Quercus macrocarpa, mossycup oak	43	20	41 0	2.0	26.2	34.2	23.6	9.8	6.0	None	None	8 3	12 36
Rhamnus cathartica, buck thorn	38	15	18 6	1.2	37.4	37.4	24.1	1.2	None	None	None	4 2	4 16
Salix britzensis, bronze golden willow	10	23	37 4	1.6	30.2	26.3	23.3	15.5	4.4	None	None	7 0	4 36
Salix vitellina, golden willow	42	30	47 0	1.5	28.4	34.7	24.5	12.2	None	None	None	6 8	6 36
Shepherdia argentea, silver buffaloberry	12	12	22 0	1.8	23.0	26.0	23.3	19.3	6.6	1.6	None	5 10	6 18
Syringa vulgaris, lilac (common)	30	15	24 0	1.6	28.5	26.0	21.2	14.4	9.6	None	None	5 0	12 30
Tamarix pentandra amurensis, Tamarix	10	14	9 6	.7	35.5	19.5	28.0	11.2	5.6	None	None	6 8	48 60
Tilia americana, American linden	28	30	27 0	.9	11.9	20.7	28.8	21.3	10.1	7.1	None	5 3	12 36
Ulmus americana, American elm	40	36	63 0	1.7	36.6	32.8	24.3	6.0	None	None	None	4 0	6 18
Ulmus pumila, dwarf Asiatic elm	12	26	27 0	1.0	22.6	40.3	30.4	6.5	None	None	None	4 2	12 24
Mean				1.3	31.1	34.4	22.6	9.1	2.1	34	.06		

The data in table 1 show that 97.3 percent of the roots growing in Fargo clay were confined to the upper 4 feet of soil. The deepest penetration of any root—that of Hibernal apple—was 10¼ feet, and the slightest—that of butternut—was 2⅔ feet. The average of all species gave a mean maximum root length of 1.3 times the tree height. Blue spruce (with 0.4) among the evergreens, and *Tamarix pentandra* (with 0.7) among the deciduous trees had the smallest relative spread; while jack pine (with 1.9) among the evergreens, and chokecherry and black walnut (with 2.1 each) among the deciduous plants had the largest, (fig. 1, *B–O*).

Ten Eyck (*15*) found, in a study of the root systems of cultivated plants grown at this station in the same type of soil, that Rural

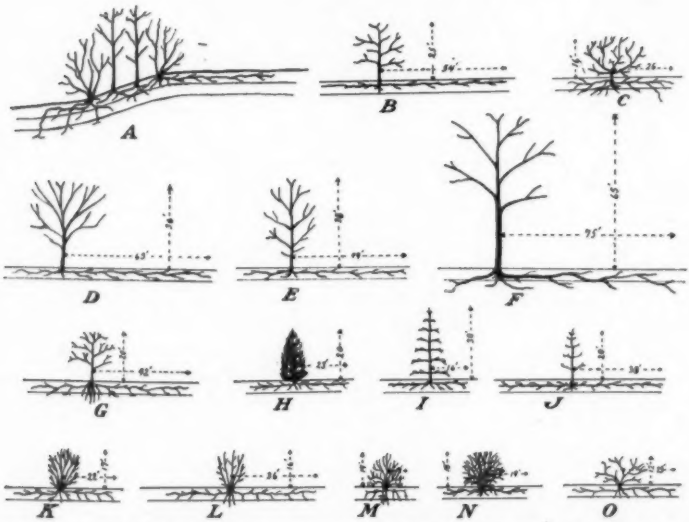

FIGURE 1.—Diagram showing distribution of tree and shrub roots in Fargo clay. (In the case of wide-spreading plants, roots are shown fully in one direction only. Lines beneath the soil level indicate 5-foot depths). *A*, cross section of four tree rows, on a slope from a dry area into a moist one. Two rows of green ash grow in the middle with one row of golden willow on each side. Note the wider spread and shallower rooting of willow on the dry soil as compared to that on the soil which in normal years is flooded each spring. *B*, black walnut, *Juglans nigra*. *C*, hibernal apple, *Malus sylvestris*. *D*, American elm, *Ulmus americana*. *E*, green ash, *Fraxinus lanceolata*. *F*, northern cottonwood, *Populus monilifera*. *G*, mossycup oak, *Quercus macrocarpa*. *H*, Colorado juniper, *Juniperus scropulorum*. *I*, Colorado spruce, *Picea pungens*. *J*, jack pine, *Pinus banksiana*. *K*, Siberian pea-tree, *Caragana arborescens*. *L*, Chokecherry, *Prunus virginiana*. *M*, Amur tamarix, *Tamarix pentandra amurensis*. *N*, buck thorn, *Rhamnus cathartica*. *O*, Silver buffaloberry, *Shepherdia argentea*.

potato roots penetrated 3 feet deep, sugar beets 3½ feet, native grasses 4 feet, flax 2½ feet, and *Bromus inermis* at least 5½ feet and probably more. Weaver (*16*), in Nebraska, found that wild alfalfa penetrated 16½ feet, buffalo grass 7 feet, wheat 3 feet, corn 8 feet, and alfalfa 20 feet. Horseradish is reported (*17*) as penetrating 14 feet deep, carrots 8 feet, and squash as having a 17-foot spread of roots with a 6-foot downward penetration. Thus, it would appear that tree roots occupy much the same soil layers as do ordinary field crops.

To compare the results obtained on the heavy clay soil with uncertain drainage, characteristic of the conditions at Fargo, another

series of observations was made near Buffalo, N. Dak., 38 miles west of Fargo. Buffalo lies just outside the Red River Valley, and the soil, a Barnes loam, is lighter and more sandy, and except for the greater organic content near the surface, is uniform far below any depth reached in the Fargo studies. Trees of the following species were excavated and studied: Golden willow, green ash, northern cottonwood, boxelder, and soft maple. It was found that the depth of penetration and the spread of the roots in this soil were practically the same as in the heavy soil; perhaps a few inches deeper, but not enough to affect the general conclusion. Data on golden willow and green ash are given in table 2.

TABLE 2.—*Effect of soil type and moisture on the roots of golden willow and green ash*

Foot of depth and diameter of root (inches)	Roots in 50-foot trench							
	Golden willow				Green ash			
	Sandy soil		Heavy clay soil		Sandy soil		Heavy clay soil	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
First:								
0–¼	290	83	92	64	105	252	60	70
¼–½	3	10	25	12	0	28	9	8
½–1	3	4	2	0	20	8	9	0
1–2	0	0	4	0	5	8	0	2
Above 2	3	2	0	4	0	16	0	2
Second:								
0–¼	135	94	118	96	170	164	72	93
¼–½	6	6	22	20	5	16	6	15
½–1	3	0	10	12	5	8	15	4
1–2	0	0	0	4	0	4	3	2
Above 2	0	4	0	0	0	12	0	0
Third:								
0–¼	54	176	92	104	145	152	111	260
¼–½	0	6	10	4	0	8	9	6
½–1	0	2	4	4	0	0	6	6
1–2	0	0	0	0	0	0	0	2
Above 2	0	0	0	0	0	4	0	2
Fourth:								
0–¼	27	134	53	136	55	116	52	35
¼–½	0	2	0	12	0	0	0	6
½–1	3	4	0	0	0	0	0	1
1–2	0	0	0	0	0	0	0	0
Above 2	0	4	0	0	0	0	0	0
Fifth:								
0–¼	24	64	0	84	0	28	0	75
¼–½	0	0	0	4	0	0	0	4
Sixth:								
0–¼	0	94	0	92				
Seventh:								
0–¼	0	28	0	24				
¼–½	0	0	0	8				
Eighth:								
0–¼	0	6	0	0				
Ninth:								
0–¼	0	2	0	0				
Tenth:								
0–¼	0	2	0	0				

Maximum depth reached by traced roots of golden willow in heavy clay soil: Dry 6 feet 8 inches; wet 14 feet.

EFFECT OF SOIL MOISTURE ON ROOTS

Around the edge of the tree planting at Fargo there is a slough which is dry during and greater part of the year, but during the spring of most years water stands in it for such long periods that it

is not suitable for ordinary crops. Four rows of trees, spaced 10 feet apart, have been planted at the edge of the wet area as a windbreak for the horticultural plots. The 2 middle rows are green ash and the 2 outside rows are golden willow. Excavations were made in the low area and the distribution of roots growing under these moist conditions were compared with those on higher ground where the only moisture available was from the rain that fell there.

In the case of the site near Buffalo, although the soil profile is relatively uniform throughout, the surface contour is somewhat irregular; that is, there are places into which the water from melting snow and flood water runs each spring, and other high points from which any excess surface water runs off. Excavations were made both on these high points and in the low places, and the roots of such trees as were available were studied. Table 2 presents the findings with respect to golden willow and green ash under the two moisture conditions.

Table 2 shows that the willow rooted very much more deeply in both the sandy soil and clay soil when extra moisture was available. Figure 1, *A*, shows a diagram of a cross section of the windbreak at Fargo running from the high land into the flooded area. This diagram illustrates the difference in depth of rooting under the two conditions and also the much greater spread of the roots near the surface where the soil is dry. These deep roots of the willow were found to penetrate almost straight down until they reached their maximum depth, where they spread out abruptly. Moisture studies showed that at this point, where a 7-foot layer of hard shale changed to a softer layer, there was 10 percent more moisture than at the top of the layer of shale.

Table 2 also shows the difference in the rooting of green ash under the two conditions of soil moisture. Here, again, it will be noted that where the soil receives excess water the roots penetrate deeper, although they do not reach the depth attained by those of the willow. A similar condition was found with respect to cottonwood, soft maple, and boxelder in the two locations on sandy soil. Trees of these species were not available for a similar comparison in the heavy soil. This deeper penetration where extra water is available corresponds closely to the finding of Weaver (*16*) that wheat roots grown without irrigation penetrate to a depth of 3 feet and where irrigated to 5 feet, and that rye growing in dry ground penetrates to 5 feet and in moist soil to 8 feet. Shallow wide-spread root systems in semiarid regions permit trees to utilize deficient natural rainfall more fully since, as reported by Finnell (*4*), little of the water reaches the subsoil.

These root studies indicate that many of the trees which are known to be none too resistant to drought have a greater tendency to form deep, penetrating vertical roots in moist locations than do most of the drought-resistant forms. Soft maple produces deep vertical roots occasionally. Cottonwood and Northwest poplar (a supposedly hybrid species) both form such roots occasionally though none of these produce a deep-penetrating root system in anything like the profusion that the golden willow, bronze golden willow, and white willow do when moisture is abundant. Known drought-resistant trees such as oak and choke cherry, which have comparatively deep root

systems, differ from those without such drought resistance in that the deep roots are characteristic of these two species regardless of moisture conditions.

SUMMARY

At Fargo, N. Dak., which has an average rainfall of 22.34 inches, a study involving 31 species of trees and shrubs showed more than 97 percent by number of the roots to be in the first 4 feet of soil.

Without irrigation, and if permitted to spread, the roots extend horizontally from 0.4 to 2.1 times the height of the tree, depending upon the species.

When additional water, but not an excessive quantity is supplied, tree roots spread less and penetrate deeper. The effect of the additional moisture is greater on some species than on others.

The general distribution of the tree roots was much the same in clay as in sandy soils.

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GERMINATION STUDIES ON AGED AND INJURED SEEDS [1]

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INTRODUCTION

From 1909 to 1933, various investigations upon problems of germination were carried on at the North Dakota Seed Laboratory by the writer and his assistants and by advanced students under the writer's supervision. The present paper includes results of some of the experiments that deal with special conditions of seeds.

CLOVER AND ALFALFA

LONGEVITY OF NORMAL SAMPLES

Some of the first studies dealt with the seeding value of "hard" seeds in clovers. Much has been published on this subject since the results of these studies were reported, and only certain features need to be considered here. In one of the first experiments six samples of alfalfa (*Medicago sativa* L.) and two each of sweetclover (*Melilotus alba* Desr.) and red clover (*Trifolium pratense* L.) were tested every 2 months for hard-seed content. These samples, assembled in the early winter of 1913–14, have been continued as a longevity trial, and 20 years' data are now available. The samples were kept in ordinary manila seed envelops, stored in ventilated galvanized boxes which were kept in the laboratory either on or near the floor. Germination tests were made between blotters by standard methods, and 500 seeds were used for each test during the first season.

The samples for the trials were selected with much care. The first three of alfalfa shown in table 1 were grown in western North Dakota in 1913, no. 14497 was grown in Montana, and no. 14469 in South Dakota. No. 14487 was imported Turkestan seed which came from a wholesale seed house and showed the typical color and intermixture of foreign seeds. Both lots of sweetclover were secured from seed houses, the crop being new at that time and no locally grown seed available. No. 14295 was from a St. Louis dealer and no. 15093 was said to be Kansas-grown. No. 14295 was rather immature and contained after recleaning, about 30 percent of greenish-colored seeds. Separate tests showed a germination of 38 percent and 29 hard for these green seeds as compared with 66 and 33 hard for the yellow. The red clovers were grown in Cass and Traill Counties, respectively, in North Dakota. A note in the original entry states: "All lots containing trash and light, shrunken seeds were blown in the vertical air blast separator to grade them evenly." Notwithstanding this, the samples are not quite so carefully prepared as would be desired by present standards.

[1] Received for publication June 11, 1935; issued February 1936. Paper no. 15, Journal Series, North Dakota Agricultural Experiment Station.

TABLE 1.—*Germination and percentage of hard seed in various samples of alfalfa, sweetclover, and red clover seed during the first year of storage*

Sample no.	Percentage of germination and of hard seed in—											
	February		April		June		August		October		December	
	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed
Alfalfa:												
14358	64	33	62	33	77	21	76	21	81	15	89	8
14397	59	38	59	33	57	27	66	28	79	18	81	14
14407	67	32	67	27	78	19	79	20	84	12	84	12
14497	79	16	79	16	83	14	85	13	87	10	91	7
14469	96	3	91	6	94	5	96	3	96	3	96	2
14487	95	4	95	2	93	3	96	2	95	1	94	3
Sweetclover:												
14295	59	35	57	33	58	31	64	23	60	29	63	25
15093	68	27	70	26	69	26	69	26	71	22	69	27
Red clover:												
14446	62	35	57	36	67	29	71	22	69	26	69	28
14448	83	12	83	8	91	7	90	7	91	6	92	4

After the first year, only 200 seeds were used in each test as it was felt that the variations would be somewhat smaller and that these samples would give the significant differences. In general, differences of less than 5 percent are not considered significant unless they appear consistently through a considerable number of tests. The causes and extent of variation have been discussed by the writer in another paper (*8*).[2]

Germination tests, although carried out under standard conditions in standard chambers, are subject to some variations, especially in respect to moisture. It is believed that the common legume seeds are as little sensitive as any kinds of seeds and that this factor is relatively unimportant. The personal factor, however, is important in longevity trials. The actual work on these samples was done by six or more different workers as the laboratory staff changed from year to year, but at all times the tests were under the supervision of the writer. More important than the personal element is the fact that old seeds differ from new both in behavior and appearance. This makes it more difficult in the case of old seeds to determine "normal" germination, "hard" seeds, etc. Even if the work is done by the same person each year, his standards may change unconsciously. Seed laboratory methods, especially the interpretation of germination tests, have been under constant scrutiny in an effort to secure more nearly uniform procedure. Some soil tests of these samples have been made but not regularly, and a field trial of 4 of the alfalfas and 1 sweetclover is reported in connection with other trials (p. 1098). The foregoing discussion applies to the data in tables 1 and 2, and also to other trials to be described in later paragraphs.

A summary of the results of the germination tests with alfalfa, sweetclover, and red clover is given in table 1. It will be noted in table 1 that the alfalfa which began with a fairly high percentage of hard seed showed a well-marked decrease by June, and a still further decrease by late fall. A similar, but less marked effect, appears in the red clover. One sweetclover sample showed practically no change,

[2] Reference is made by number (italic) to Literature Cited, p. 1106.

while the other showed a decrease in hard seeds but without a corresponding increase in germination. A test of all samples in June 1915 following gave practically the same results as the December test. A summary by 5-year periods is shown in table 2.

TABLE 2.—*Germination and percentage of hard seed in various samples of alfalfa, sweetclover, and red clover seed by 5-year* [1] *intervals over a period of 20 years*

Sample no.	Percentage of germination and of hard seed in—									
	1914		1920		1924		1929		1934	
	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed
Alfalfa:										
14358	64	33	70	1	69	2	55	0	53	2
14397	59	38	72	3	68	3	71	3	64	4
14407	67	32	79	3	72	5	65	3	55	3
14497	79	16	53	1	59	5	53	2	54	1
14469	96	3	81	2	78	2	81	1	82	1
14487	95	4	95	0	86	0	85	0	75	0
Sweetclover:										
14295	59	35	32	30	28	23	22	21	26	18
15093	68	27	71	16	69	15	58	14	48	22
Red clover:										
14446	62	35	56	27	46	14	32	14	16	17
14448	83	12	40	13	39	4	17	3	7	6

[1] No test was made between 1914 and 1920.

Table 2 indicates (1) almost complete loss of hard seed in the alfalfa during the first 5 years, but a long retention of a small percentage; (2) retention of over 50 percent of viability in the same samples at 20 years; (3) retention of 75 to 80 percent of viability in two samples of alfalfa which contained practically no hard seeds at the beginning; (4) a much slower decrease of hard seeds and about equal decrease in germination in the sweetclover, the total loss being greater than in the alfalfa; (5) a still more rapid decline in red clover to a very low germination at 20 years; and (6) a marked difference in the behavior of different samples of the same kind of seed.

The finding of an exceptionally high retention of viability in two samples of alfalfa which had a low content of hard seed at the beginning of the experiment is perhaps surprising, and emphasizes the individuality of samples. Since the hard-seeded condition in alfalfa is rather temporary, there seems little reason to expect that it would affect the longevity. Where this condition is of long duration as in sweetclover, we naturally expect the permeable seeds to lose their vitality first, the hard seeds retaining theirs at least until the coats become permeable.

The first experiment had shown a marked decrease in hard seeds in alfalfa samples during the first year, but more frequent tests seemed desirable. A new series of samples which had been received from November 1914 to April 1915 was assembled and tested in the same way the 1st of each month from April to October. These were fresh seeds all with a high percentage of hard seeds and grown mostly in North Dakota (1 Minnesota, 2 Montana, 1 unknown). The averages of the results are given in table 3.

TABLE 3.—*Germination of and percentage of hard seed in various samples of alfalfa, sweetclover, and red clover seed between April and October*

Seed	Percentage of germination and of hard seed in—									
	April		May		June		July		October	
	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed
Alfalfa (8 samples)	43	55	59	39	63	35	66	32	72	26
Sweetclover (3 samples)	30	66	36	60	33	63	38	59	33	60
Red clover (3 samples)	60	39	62	36	65	34	64	35	64	35

These results bear out those of the first trial, in showing a sharp decrease in the percentage of hard seeds in the alfalfa in early spring and a continued decrease at a slower rate during the rest of the summer. The sweetclover and red clover showed practically no change during the year. Retained for 15 years longer, the samples have

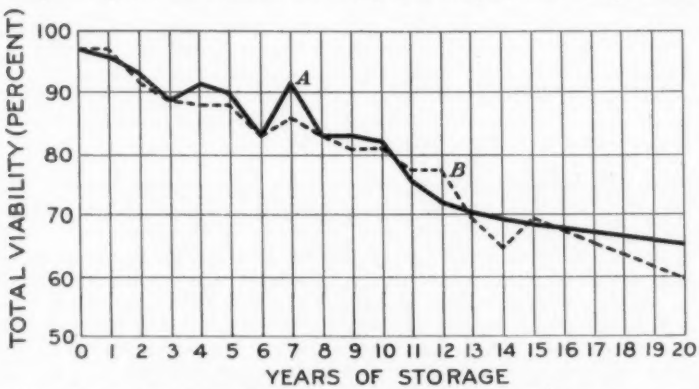


FIGURE 1.—Results of combined germination tests of all samples of alfalfa (*A*) and sweetclover (*B*) after various periods of storage. No data for the years 16 to 19, inclusive.

shown decreases comparable to those in table 2, again with considerable differences between apparently similar samples.

A third similar lot of samples was selected in 1920 for a scarified seed test. The unscarified portions of these samples were tested each year until 1930. By combining all the data available for the different lots of normal alfalfa and sweetclover seed, and considering only total viability (germinating plus hard), the graph shown in figure 1 was secured.

The results agree in general with those found by Sifton (7), but indicate considerably higher retention of viability. Possibly this is due to the fact that samples containing a high percentage of hard seeds were used for the most part. However, the highest germination of all samples at the age of 20 years was from two samples of alfalfa which contained very few hard seeds. Sifton has called attention to the rather sudden loss of vitality in the cereal seeds after about the tenth year, as compared with a gradual decline in the clovers. The

different behavior of clovers is possibly accounted for by the fact that they have a less uniform maturity, are subjected to a greater degree of injury in threshing, and undergo a gradual loss of impermeable seed coat.

LONGEVITY OF SCARIFIED SEEDS

The longevity of scarified sweetclover seed has been discussed quite fully in another paper (*11, p. 13*). The behavior of scarified alfalfa seed is essentially similar. The scarified seeds lose their vitality in the course of 1 to 2 years, probably as a result of increased oxidation permitted by the broken seed coats, and the loss of vitality in the remaining seeds of the sample then proceeds as with unscarified seeds. Different samples may behave quite differently owing to variations in degree of scarification or other abrasions in threshing, or to degree of maturity and other natural influences. The fact that the hard seeds of alfalfa become permeable to a large extent during the spring of the first year, and that they will germinate better in the field than in the laboratory, has practically closed the question of scarification so far as alfalfa is concerned.

GERMINATION OF FROSTED SEEDS

The germination of the immature seeds of sweetclover and of those discolored by frost has already been discussed (*11*). Immature seeds of alfalfa are relatively rare, but a small percentage of brown seed is very commonly present and is an important factor in the commercial value of the seed. The crop of 1925 contained an unusually large amount of seed discolored by frost. The germination of the brown seeds was found to be quite good if they were plump and of good weight, but it seemed desirable to determine whether such seeds would retain their viability. A series of 5 samples of alfalfa and 2 of sweetclover, all North Dakota grown, were assembled. All samples were carefully recleaned and approximately 20 g of each was separated by hand into brown and yellow, and stored in separate packages. The character of the samples is indicated in table 4.

TABLE 4.—*Quality of North Dakota grown alfalfa and sweetclover seed samples as affected by the presence of brown seeds discolored by frost*

Seeds and sample no.	Place grown in North Dakota	Percentage brown (by weight)	Weight of 200 seeds	
			Yellow	Brown
Alfalfa:			*Grams*	*Grams*
47581	Hastings	34.8	0.360	0.340
48248	Washburn	37.4	.420	.380
48770	Arnegard	41.2	.405	.360
48828	Washburn	46.2	.415	.380
49053	Lark	39.4	.415	.395
Sweetclover:				
47450	Christine	51.5	.390	.370
48034	Horace	31.8	.420	.385

The results of these tests were somewhat more difficult to interpret than those of the previous experiments because the condition of "hard" seeds is not so easily recognized in the brown condition. In general it may be said that during 6 years of storage the average actual germination of the yellow seeds in the alfalfa remained constant ta about 57 percent, while the hard seeds decreased from 25 to 12

percent. In the brown seeds, germination decreased from 54 to 30 percent, while the hard seeds remained practically constant at about 13 percent. In the sweetclover, germination decreased from about 70 to 50 percent in both yellow and brown seed, and the hard seeds remained essentially constant at about 30 and 25 percent, respectively.

These results differ from those previously obtained by the writer and by other workers in indicating a longer retention of the hard-coated condition by frosted seeds. As previously suggested, it was felt that there was some question about the brown seeds recorded as hard at the end of the usual test. In 1935 the hard seeds remaining at the end of the test were carefully reconsidered, but without any material change in the results. All of the remaining hard seeds were then scarified by rubbing with emery cloth and replaced in the germination chamber. In two of the alfalfa samples (nos. 48248 and 48828) about two-thirds of the brown hard seeds softened without producing normal sprouts. In the other samples this result was less marked, though in all samples there were more dead brown seeds than yellow ones. The average additional percentage of dead seeds added to the original result by scarifying the hard seeds was 15 and 3 for the brown and yellow sweetclover and 7.5 and 1 for the alfalfa, respectively. It thus appears that in some of these hard brown seeds the vitality of the seed had been destroyed without rendering the coat permeable. Since no test of this sort had been made previously it is not known whether this occurred in the first instance or during storage.

FIELD GERMINATION OF CLOVERS

Of various field trials, two were quite extensive and of special interest since they included aged and injured seeds. In 1927, Wm. M. Jackson, at the Ellendale (N. Dak.) Normal and Industrial School, carried out a series of tests outlined by the writer. Plantings were made on May 6, May 26, June 22, and July 13. The first two were the most successful. In the latter two germination was poorer and weeds were more troublesome. A total of 45 carefully selected samples was used in the following groups: (1) Check—1 alfalfa, 3 sweetclover; (2) hard seeds—4 alfalfa, 3 sweetclover; (3) broken (chipped and cracked) producing broken sprouts—5 alfalfa, 4 sweetclover; (4) immature—5 alfalfa, 4 sweetclover; (5) frosted—3 alfalfa, 2 sweetclover; (6) old—4 alfalfa, 1 sweetclover.

For each planting 200 seeds were used. The old seeds were from the same samples shown in table 1, and therefore were 14 years old. The frosted seeds were from the samples listed in table 4, and were only 1 year old. The average weight of the seeds in the immature lots was 60 to 70 percent of that of normal seeds. The samples with broken seeds produced from 18 to 59 percent of broken sprouts in the blotter tests. While these are not counted as germinated, it was suspected that there were other sprouts which would not succeed in producing plants. Expressing the results in terms of percentage of plants in the field to germination in blotters the averages for all four plantings were:

(1) Checks—alfalfa 64, sweetclover 51.
(2) Hard seeds—alfalfa 123, sweetclover 48.
(3) Broken seeds—alfalfa 42, sweetclover 24.
(4) Immature—alfafa 23, sweetclover 23.
(5) Frosted—alfalfa, yellow 59, brown 38; sweetclover, yellow 53, brown 40.
(6) Old—alfalfa 41, sweetclover 37.

The check sample of alfalfa showed about 15 percent of hard seed in the blotter test, the sweetclovers less than 10 percent. In series 2 about 65 percent of hard seeds were present in both alfalfa and sweetclover, and here the alfalfa produced more than twice as many plants as the sweetclover; that is, a large number of the hard seeds of the alfalfa produced plants and apparently none or few of the sweetclover. In the other lots, normal seeds produced about half as many plants as in the blotter test, the old and discolored seeds 10 to 15 percent less, and the immature and broken seeds about one-fourth as many. The marked difference between the alfalfa and sweetclover in the broken seeds is probably due to the difference in behavior of the hard seeds (35 percent present).

A second and similar trial was carried out at Fargo, N. Dak., in 1928 by W. A. Davidson. The same samples were used for the old and discolored seeds, but for the other lots new samples were employed similar to those used in the previous trial. Plantings were made on May 22 and July 9. The first gave better results. The soil was very dry at the time of planting, but rain fell on June 7 and the soil was in good condition at the time of the second planting. The results were similar to those at Ellendale and calculated on the same basis were:

(1) Checks—alfalfa 48, sweetclover 62.
(2) Hard seeds—alfalfa 146, sweetclover 61.
(3) Broken seeds—alfalfa 22, sweetclover 32.
(4) Immature—alfalfa 24, sweetclover 27.
(5) Frosted—alfalfa, yellow 70, brown 40; sweetclover, yellow 63, brown 36.
(6) Old—alfalfa 46, sweetclover 33.

A field planting was made May 8, 1920, of machine-scarified and of unscarified samples from identical lots of fresh seed. Calculated as in the preceding paragraph, the results were:

Alfalfa, 5 samples—unscarified, in field, 147; scarified, germinated between blotters, 198, in field 181.

Sweetclover, 3 samples—unscarified, in field, 82; scarified, germinated between blotters, 148, in field 104.

Red clover, 1 sample—unscarified, in field, 73; scarified, germinated between blotters, 102, in field 67.

In the 1920 planting, the seedlings were removed as they appeared and the experiment was carried on for only 1 month. In the other two plantings, the plants were left during the summer. On the whole, the results are in fair accord with those of other workers, although they do not indicate the germination of hard seeds later in the season as reported by Leggatt (*3*) and Whitcomb (*13*), nor their better germination at higher temperatures as reported by Lute (*4*). The influence of the variable factors of soil and weather are obviously very great. The writer wishes to stress particularly the variability in condition of samples. A classification into "scarified" and "unscarified" often is meaningless because of the variable effects of the scarifying process. This has been shown by the writer (*11, p. 15*) in a study of sweetclover.

LONGEVITY OF SOYBEANS

A collection of 23 samples of soybeans (*Soja max* (L.) Piper) tested for the Department of Agronomy of the North Dakota Experiment Station in 1924, was again tested in 1926 and 1928, and a few were tested in 1929–31. One hundred seeds were used for each test. The crop years of 1921, 1922, and 1923 and seven varieties were represented. The combined results are presented in table 5.

TABLE 5.—*Viability of soybeans of the crops of 1921–23 after storage in laboratory, 100 seeds being used per test*

Item	Results after storage for indicated number of years								
	1	2	3	4	5	6	7	8	9
Samples.....number..	13	4	17	4	17	8	8	4	3
Average germination.....percent..	92	91	83	84	67	65	51	47	30

Four of these samples gave 80 percent or more germination in 5 years, while only two had dropped below 50 percent. One sample of the Chestnut variety gave 90 percent the eighth year, but fell to 47 percent the tenth year (the only record for the tenth year). One sample of Manchuria gave 90 percent the seventh year but dropped to 66 percent the eighth year. The Wisconsin Black variety usually has shown considerable hard seed in fresh samples, but the two lots of it in this series did not hold their viability as well as some other varieties. In 1931, three of the best and three of the poorest samples were planted in the garden. The results are shown in table 6.

TABLE 6.—*Comparison of laboratory and field germination of old samples of soybeans of different varieties*

Variety	Age	Germination in laboratory	Germination in field
	Years	*Percent*	*Percent*
Chestnut	10	47	39
Manchu	9	33	5
Manchuria	8	66	44
Mandarin	9	21	22
Manchuria	--	14	4
Minsoy	8	16	10

The results of these tests are in fair agreement, considering the small number of trials, and are sufficient to show that the samples were all capable of producing plants in the field. It may be mentioned that some of them are still continuing (1935) to produce volunteer growth each year as a result of spontaneous reseeding.

GERMINATION OF BROKEN SEEDS IN FLAX

The broken seeds of flax (*Linum usitatissimum* L.) present a considerable problem, as may be seen from the fact that all samples examined at the seed laboratory from the crop years 1931 and 1932 contained an average of 4.5 percent by weight of pieces comprising not more than one-half of a seed. This includes samples of all kinds, cleaned and uncleaned. Occasionally the amount runs as high as 15 percent. The seeds may be broken in various ways, but reference is made especially to those that are broken transversely. In determinations of purity, according to the rules of the Association of Official Seed Analysts of North America (*12*), pieces of not more than one-half the size of a seed are placed in "inert matter", while pieces exceeding one-half are placed in "pure-seed." Although this procedure results in placing pieces from the larger end of the seeds

which contain no plumules with the seeds used for germination, it has been retained as the simplest working basis for seeds of most species.

From the 1930 crop, 11 cleaned samples were selected for germination experiments. All were of the Bison variety, grown in seven different counties, well distributed over North Dakota. Each sample was separated carefully into five divisions which gave an average composition in percentage by weight as follows: (1) Perfect seeds, 80.5; (2) slightly cracked, 6.8; (3) only small pieces broken off, 4.3; (4) plumule ends; more than one-half of a seed, 1.8; (5) plumule ends, less than one-half, 2.3. The separation into the five groups involved a considerable element of personal judgment, particularly in group 3, where minute pieces of seed coat may be broken from the plumule end in as much as 20 to 30 percent of the seeds. Such seeds were not used. The separations were made on 20 g by a single experienced worker after various comparative trials had been made by different workers and many pieces had been weighed. Seeds were tested in soil in the germinating chamber and a field planting was made about May 20. The soil in the field plot was firm and in good condition, and although the weather turned unusually warm a few days after the seeds were planted, germination and growth were good. The counts recorded in the field are of plants finally produced. The results of the tests are shown in table 7 and figure 2.[3]

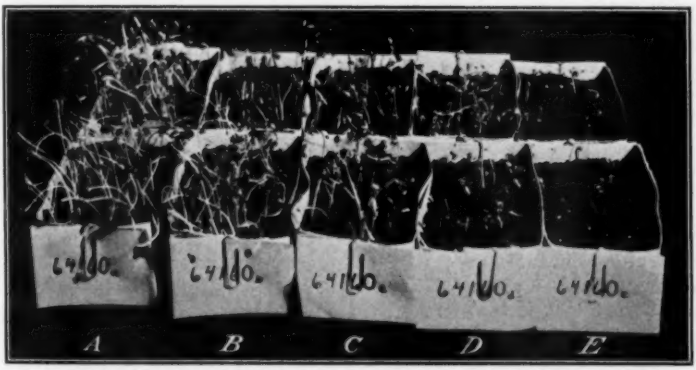


FIGURE 2.—Germination of normal and of broken seed of flax in soil in the germinating chamber, showing sample no. 64160, in front, and no. 63473, behind: *A*, Normal seed; *B*, slightly cracked seed; *C*, seed with only small pieces broken off; *D*, plumule ends, more than one-half of a seed; *E*, plumule ends, less than one-half of a seed.

It was somewhat of a surprise that even a few plants were produced by the small pieces. The variation in the samples in the field was striking, but it seemed to be mainly accidental as similar differences were not found in the soil tests in the chamber. No other sample gave such a high percentage of plants in the field from the normal seeds as no. 64160, yet all of the broken lots from this sample gave very poor results. The behavior of this sample in the chamber

[3] Special credit is due Isabel Barrett for the laboratory work on this experiment as well as for work on other projects over several years. Charlotte and Anita Mary Blake did a considerable amount of the work on the earlier projects.

was quite similar to that of no. 63473; in fact it gave the highest number of sprouts from group 2.

TABLE 7.—*Germination (percent) of perfect and of broken flaxseed in soil in the germination chamber and in the field*

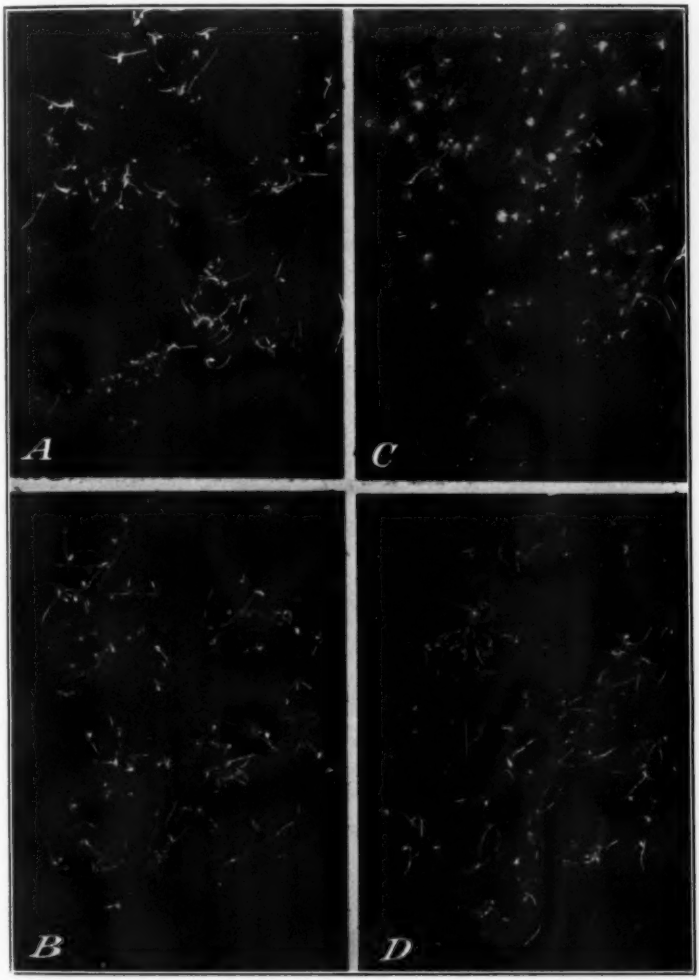
Sample no.	Perfect seed		Cracked seed		Slightly broken seed		More than half a seed		Less than half a seed	
	Chamber	Field	Chamber	Field	Chamber	Field	Chamber	Field	Chamber	Field
64160	97	90	86	12	73	7	51	0	9	0
63473	91	69	64	52	55	50	48	19	28	7
Average of 11 samples	89	63	73	30	57	20	38	8	13	3
Weak growths	2	.....	7	.....	11	.....	17	.....	15	.....

GERMINATION AND LONGEVITY OF HULLED TIMOTHY AND OTHER HULLED SEEDS[4]

Several workers who have reported (*1*, *2*) on the relative germination of hulled (naked caryopses) and unhulled (enclosed by lemma and palea) grains of timothy (*Phleum pratense L.*) found a considerably lower average germination for the hulled grains, but they did not carry the study further. McRostie (*5*) concluded (on the basis of one lot only?) that no serious deterioration should take place in 3 or even 4 years of storage. Newton and Ficht (*6*) found a rather rapid decline in germination of the hulled grains, but this was not reflected in the field because the rate of seeding was sufficiently heavy to provide enough plants. From their germination tests the one lot used appears to have been of representative character. The present writer reported (*10*) upon a survey of 77 samples received in the year 1923–24. From these, 10 samples which contained from 36 to 63 percent of hulled grains were selected and stored with the samples of clovers already discussed. These have been tested in May of each year, using each time 200 each of hulled and unhulled, and placing the separations from a given sample on the same blotter in the chamber. Only samples which showed a high initial germination of the hulled grains were used. The average initial germination was 95.2 for the hulled and 98.6 for the unhulled.

Figure 3 shows that the viability of the unhulled seeds remained essentially unimpaired for 5 years, after which it declined with increasing rapidity. The hulled grains lost viability steadily from

[4] In North Dakota and neighboring States the term "hulling" is in common use for the process of removing the covering (pod, pericarp) from the seeds of legume forage crops. Especially in sweetclover, the seeds are termed "unhulled" in their original condition and "hulled" after the pericarp has been removed. It should be noted that in this sense the term "hulled" refers to the result of a mechanical operation. Scientific writers have often described the grains of grasses as "hulled" when normally enclosed by the lemma and palea after threshing. "Hull-less" varieties are those which normally are freed from the lemma and palea in threshing. The naked condition in timothy and in the common varieties of millet is abnormal. Perhaps "deglumed" would be a better term for these, but it appears impossible to establish a simple and precise terminology for so many variants. The usage employed in the present paper is that commonly used commercially.



Germination of hulled and unhulled timothy seed after 8 years' storage: *A* and *C*, hulled; *B* and *D*, unhulled; *A* and *B*, sample no. 42260; *C* and *D*, sample no. 43869. No. 42260 gave the best results for hulled seed and no. 43869 the poorest.

the first, and in the better samples the results tended to approach those of the unhulled grains. In addition to these differences there were variations between individual samples, distinct breaks occurring in various years.

Personal judgment might seem to account for some of the apparent irregularities, but these usually concerned only certain samples, no general differences appearing in all of the samples in any one year. The sprouted grains from the test in 1932 of one of the best and one of the poorest samples are shown in plate 1. A soil test of no. 42260 made in the chamber on the same date gave 40 and 83 percent

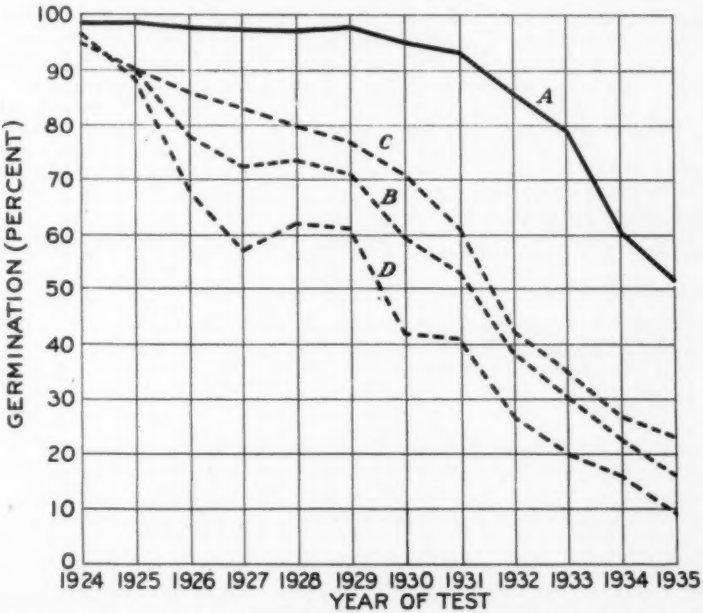


FIGURE 3.—Germination of hulled and unhulled timothy in storage. Average for unhulled (*A*) and hulled (*B*) in 10 samples; average of 6 best (*C*) and 4 poorest (*D*) of the hulled.

for the hulled and unhulled as compared with 61 and 92 for the blotter test and 89 and 98 for a soil test of a fresh check sample.

It was noted in 1934 that germination continued after the 8-day period, and when the seeds were left for 9 days more, an average additional 6 and 17 percent was recorded for the hulled and unhulled seeds, respectively. There were many grains producing shoots but no roots (therefore not counted as germinating), these averaging 5 and 6 percent, respectively, for the hulled and unhulled, but with the greatest variation in the latter. The unhulled seeds of sample no. 43869 retained their vitality best of all the samples, but the hulled seeds made one of the poorest showings. The information regarding this sample indicated that it was overripe and had stood in the shock, but was not damaged by rain.

It appears from these tests that while the hulled seeds usually germinate more poorly than the unhulled, in some samples they may be little inferior at first but lose vitality more rapidly than the unhulled. There may even be wide differences in rate of loss between the two kinds in one sample.

For several years comparative tests have been made whenever samples were available of the germination of the naked caryopses of grains which normally remain enclosed by the lemma and palea, particularly those of emmer (*Triticum dicoccum*) and quackgrass (*Agropyron repens*). The results are summarized in table 8.

TABLE 8.—*Germination of various seed in hulled and unhulled condition*

Kind of seed	Samples	Average content of hulled seed	Average germination	
			Hulled	Unhulled
	Number	*Percent*	*Percent*	*Percent*
Oats (*Avena sativa*)	12	13	81	97
Emmer (*Triticum dicoccum*)	1	(1)	18	74
	1	20	98	95
Proso millets (*Panicum miliaceum*)	19	22	51	94
Foxtail millets (*Setaria italica*)	9	18	53	91
Bromegrass (*Bromus inermis*)	1	10	72	95
Reed canary grass (*Phalaris arundinacea*)	1	37	16	78
Quackgrass (*Agropyron repens*)	1	100	5	----
	1	100	31	----
	1	70	24	88
	1	93.6	15	91
Yellow bristle grass (*Setaria lutescens*)	4	100	24	----
Green bristle grass (*Setaria viridis*)	13	56	46	86
Buckwheat (*Fagopyrum esculentum*)	1	3	98	98
Wild buckwheat (*Polygonum convolvulus*)	6	----	27	17

[1] Old and moldy.

In these tests it was found that the hulled seeds germinated more poorly, but with large variations, some samples showing no differences between the hulled and the unhulled. The hulled oats varied from 63 to 98 percent and with little relation to the amount of hulled grains present.

The millets are probably of most importance. A record has not been kept of the percentage of hulled grains in all samples, but in the proso millets it frequently reached 20. In the foxtail millets it was much lower. The bristle grasses were obtained from samples of alfalfa and sweetclover, chiefly from the 1927 crop which contained large quantities of these seeds. The lemma and palea envelop the caryopsis closely, but are frequently removed by the close hulling which the seed receives. The radicle of the embryo often is broken in the process. The yellow bristle grass, because of its larger size, is nearly always hulled and very often broken. An outstanding sample among the bristle grasses was one in which 93 percent of the grains were hulled and 87 percent of them germinated, the highest germination in the entire group. In another sample containing 50 percent hulled seed, 71 percent germinated, the second highest test, while of the unhulled seed 70 percent germinated, the lowest by 12 percent, for the group. Bristle grass germinated readily in all cases.

In quackgrass, the lemma and palea adhere tightly and the radicle projects distinctly so that it is very often broken a little. The fourth sample reported in table 8 was secured from a dealer who stated

that it came from new-crop sweetclover which had just been hulled and scarified. The first test was made April 1. On May 1 and June 1, the hulled quackgrass gave the same germination, but on December 1 it gave only 4 percent, the unhulled 91 as before.

Wild buckwheat is a common impurity, and in alfalfa or other small seeds it becomes considerably broken. The embryo lies close to the surface, on one angle, and so is likely to be injured in hulling, which in this case means removal of the pericarp. The germination tests on this seed were unsatisfactory, as the normal seeds failed to respond in 3 cases out of 4. Nor did they respond to any extent to chipping, testing in soil at 18° C., or on blotters at 14°. The ordinary buckwheat (*Fagopyrum*) is not at all comparable to wild buckwheat, for its embryo lies in the center of the seed.

LONGEVITY OF PERENNIAL SOWTHISTLE SEEDS

The achenes of *Sonchus arvensis* have germinated readily with the usual 20°–30° C. alternation as previously reported (*9*), and quite uniformly have produced about 95 percent. Many tests have been made, but no regular annual ones. Fresh seed has been collected nearly every year and as tested May 1, 1935, these lots showed a poor growth for the 1929 crop and none for the crops of 1922 to 1928.

SUMMARY

Annual germination tests were made upon various seeds stored in the laboratory up to 20 years. Under these conditions the viability of good alfalfa and sweetclover seed declined steadily from about 95 to about 60 percent in 20 years. Red clover dropped to about 10 percent.

Hard seeds in alfalfa became permeable to a large extent during the spring months, and while few hard seeds remained after 1 year, from 1 to 4 percent were present even after 20 years. In red clover there was a slower decrease in hard seeds, and in sweetclover very little, two samples retaining at 20 years one-half and four-fifths, respectively, of their original hard-seed content. Some samples which originally contained few hard seeds retained their vitality as well as others. Seeds of alfalfa and sweetclover touched with frost when maturing showed greater retention of hard seeds than did normal lots, but in some cases these did not produce normal sprouts when scarified.

In field plantings of samples containing a high percentage of hard seeds, alfalfa produced from one-fourth to one-half more plants than was indicated by blotter test, while alfalfa which did not contain a large amount of hard seeds and sweetclover both with and without hard seeds, produced only about one-half as many plants as indicated by the blotter test.

Twenty-year-old seeds of alfalfa and sweetclover, and also fresh seeds discolored by frost but of nearly normal weight, gave results in the field only slightly lower in proportion to their blotter test than the normal seeds. Broken and quite immature seeds gave only about one-half as good results.

Soybeans declined steadily but retained an average viability of 30 percent at 9 years.

Broken seeds of flax gave poor germination, but even pieces of less than one-half of a seed gave a maximum of 12 (average 3) percent of plants in the field.

Normal grains of timothy retained normal viability 5 or 6 years, then declined rapidly to 60 percent in 10 years. Hulled (deglumed) grains from the same samples declined steadily to about 20 percent in 10 years, but the better lots decreased to only 70 percent in 6 years.

Millets, especially the proso types, often had a considerable proportion of grains without glumes, and these germinated poorly. Grains of green bristle grass, found in samples of clover and deglumed through clover hulling, were similarly injured. Grains of quackgrass were commonly broken in the same process but gave as high as 30 percent germination.

Seeds of perennial sowthistle retained their viability for 5 years only. Wide differences were observed in the behavior of apparently similar samples, and the causes and extent of variations in such tests is discussed.

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THE NUTRITIVE VALUE OF SKIM-MILK POWDERS, WITH SPECIAL REFERENCE TO THE SENSITIVITY OF MILK PROTEINS TO HEAT[1]

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INTRODUCTION

In the commercial dehydration of raw liquid skim milk the immediate practical objective is the production of a dry powder that may be conveniently stored and readily transported. The agency of dehydration is heat. In the merchandising of these powders emphasis is placed upon their high nutritive value, particularly as sources of protein, of calcium, and of vitamin G. It is commonly, if tacitly, assumed that they are equal in nutritive value to the solids of raw liquid skim milk. However, there is more than a remote possibility that the heat treatment to which they have been subjected has lowered their nutritive value, although little definite evidence to this effect has been found in the literature.

In the drying of liquid skim milk, there are times when the resulting products are scorched. This scorching is caused in different ways, and under careful management it may be prevented. The point of interest here is that these scorched products are offered for sale through the various merchandising channels. In such cases there is ocular evidence that processing has changed the milk solids, but to what extent the nutritive value has deteriorated there is again no available evidence.

While many types of equipment are used in the manufacture of dry skim-milk powders, only two distinct processes of dehydration are in general use, one the roller process, and the other the spray process. Powders made by the roller process are largely merchandised as animal feed, while the powders sold for human consumption are largely prepared by the spray process. Whether the powders produced by the two processes are of equivalent nutritive value is not definitely known.

Of the 288,000,000 pounds of dry skim-milk powder produced in the United States in 1933, about 60 percent went into human consumption through such media as bakery goods, ice cream, confectioneries, and breakfast cereals. The remaining 40 percent was sold as animal feed. The former channels of consumption obviously represent the most particular and fastidious of sales distribution, in which a scorched product is frowned upon because of its off color and taste. The animal-feed buyer is not opposed to the scorched product because of color or taste, but he does, and should, view it with some doubt on the score of its nutritive value.

[1] Received for publication July 11, 1935; issued February 1936. The experimental data reported in this article have been taken in part from the thesis of B. W. Fairbanks submitted in 1935 to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of doctor of philosophy in animal husbandry, and in part from an experiment station project. The investigation carried on by the senior writer was made possible by the donation of funds and samples of skim-milk powders to the University of Illinois by the American Dry Milk Institute, Inc.

The investigation reported in the following pages was planned to solve these problems, in part at least, but mainly as they relate to the changes in the nutritive value of milk solids induced by the heat processes employed in the commercial manufacture of skim-milk powders.

REVIEW OF LITERATURE

Nevens and Shaw (*13*)[2] showed that the digestibility of the proteins of whole-milk powder was appreciably less than that of the proteins of liquid whole milk. They did not observe any significant differences in the digestibility of the proteins of whole-milk powders produced by the roller and the spray processes, although Miyawaki, Kanazawa, and Kanda (*11*) claim, on the basis of in vitro digestion experiments, that the digestibility of the proteins of roller-process powder is somewhat less than that of spray-process powder.

Chick (*2*) and Goldblatt and Moritz (*5*) compared the growth-promoting values of unheated and heated casein, obtaining no evidence of a deleterious effect of heat. In these tests the casein was heated for 36 to 72 hours at 110° to 130° C. As high-protein diets were used in both investigations, a possible slight inferiority of the heated casein may well have been masked. The more rapid resumption of growth in rats following depletion of vitamin A when unheated casein was used than when casein heated at 105° C. for 7 days was used, noted by Coward, Key, Morgan, and Cambden (*3*), may well have been a result of heat upon the casein itself rather than upon any vitamin A originally present in it. Morgan (*12*) obtained consistently lower biological values for toasted (150° for 30 minutes) than for raw casein, and in later work with Greaves (*6*) an attempt was made to trace the heat (140° for 30 minutes) deterioration to the destruction of certain of the amino acid components of casein; i. e., lysine, histidine, tyrosine, cystine, and tryptophane. From growth experiments they concluded that the nutritive value of both heated and unheated casein was improved by a supplement of cystine and that that of the heated casein (but not of the unheated casein) was improved by supplements of either lysine or of histidine; in nitrogen-balance experiments they found that the biological value of unheated as well as of heated casein was increased by a cystine supplement, while that of the heated casein was improved also by a supplement of lysine. Such results, indicating a supplementation of proteins simultaneously by more than one amino acid indispensable to life, are contrary to prevailing conceptions of the relation between the composition of proteins and their value in promoting protein synthesis in animals. Block, Jones, and Gersdorff (*1*) found that the lysine yielded by the acid hydrolysis of casein was not lowered by previous treatment of the casein with dilute sodium hydroxide or by dry heat (150° for 65 minutes).

The above-cited experiments on heated versus unheated casein are of no definite value in predicting the effect on the nutritive value of milk solids of dehydrating by the prevailing commercial processes, mainly because the time of exposure to heat is so much shorter in the latter case. Thus, in the roller process, the milk film is in contact with the drying roll for only about 4 seconds, and for only a fraction of this time is it exposed to the maximum roller temperature, estimated at

[2] Reference is made by number (italic) to Literature Cited, p. 1120.

134° C.[3] In the spray process the raw milk is preheated at 60° to 63°, concentrated in a vacuum pan, and sprayed into a current of air at temperatures ranging from 93° to 149°.

OBJECT, MATERIALS, AND METHODS

The object of the experiment was to compare the digestibilities and the biological values of the proteins of a series of skim-milk powders prepared by different methods and processes with each other and with those of a sample of raw skim milk which had not been subjected to any heat treatment whatever. Albino rats were used as subjects. Somewhat incidentally, the net-energy values of the two roller-process powders subjected to the severest and the mildest heat treatment were compared. A study was also made of the amino acids limiting the nutritive values of the proteins of the roller-process powders, for the purpose of determining the location of the heat injury.

Six samples of dry skim-milk powders were prepared in the Dairymen's League plant at Massena, N. Y., from the same supply of milk. Four of the samples were made upon twin-cylinder atmospheric rolls and the remaining two were prepared by the continuous spray process. These six samples were compared to raw liquid skim milk.

Raw liquid skim milk.—This sample was obtained from the Division of Dairy Manufactures of the University of Illinois and was drawn before any heat was applied. While this sample of skim milk was not taken from the liquid skim milk from which the powders were prepared, the discrepancy is not serious, as the proteins of skim milk are comparatively constant in amount and proportions.

Low-temperature powder, roller process (*r. p.*).—The steam gage at the rolls registered 50-pound pressure, and the film of milk delivered to the roll was thinner than in common practice. This powder was exposed to a temperature less than that commonly employed in commercial drying.

Choice commercial powder, roller process (*r. p.*).—Ordinary commercial plant procedures were employed, including 87 pounds of steam pressure and a milk film of ordinary thickness. This sample was a high-quality product, and would receive the highest grade adopted by the standards committee of the American Dry Milk Institute.

Slightly scorched powder, roller process (*r. p.*).—The steam pressure was increased to 90 pounds and a thin film of milk was delivered to the rolls. This sample was representative of the scorched milk powder frequently encountered in the trade.

Scorched powder, roller process (*r. p.*).—The knives were lifted intermittently from the rolls, so that the milk solids made more than one revolution of the rolls. The product was representative of the extreme scorching occasionally found in market samples.

Not preheated powder, spray process (*s. p.*).—The usual preheating process was omitted. Such a powder is not frequently encountered in the trade.

Preheated powder, spray process (*s. p.*).—The usual preheating process which is considered necessary for the preparation of a high quality good-baking spray-process powder was included.

[3] No estimate of the maximum temperature to which the milk film is exposed on the roll can possess a high degree of accuracy nor be generally applicable under all conditions.

All samples were shipped to Urbana in tin containers with tight-fitting lids, except the choice commercial powder, which was shipped in a well-made barrel lined with paper. On arrival the latter sample was transferred to 50-pound lard pails. All samples were stored in a dry place.

For purposes of precise description, the chemical composition, solubility, and color analysis of the skim-milk powders are given in table 1.

TABLE 1.—*Chemical composition, solubility, and color analysis of the experimental dry skim-milk powders*

Sample of powder	Chemical composition					Solubility		Color analysis		
	Dry substance	Nitrogen	Crude protein (N×6.25)	Fat	Gross energy per gram	Dry substance in solution	Nitrogen in solution	Brightness	Dominant wave length	Purity
	Percent	*Percent*	*Percent*	*Percent*	*Calories*	*Percent*	*Percent*	*Percent*	*mμ*	
Low-temperature, r. p.	96.12	5.41	33.81	0.91	4.06	71.3	34.6	84.5	600	1
Choice commercial, r. p.	98.06	5.50	34.38	.99	4.02	67.7	32.4	84.6	560	23
Slightly scorched, r. p.	99.87	5.41	33.81	1.07	4.02	62.4	27.9	77.1	580	17
Scorched, r. p.	98.90	5.55	34.69	.90	4.25	64.6	24.9	62.0	587	18
Not preheated, s. p.	98.32	5.40	33.75	.69	4.13	95.1	92.3	95.5	590	1
Preheated, s. p.	98.40	5.50	34.38	.62	4.12	92.6	91.6	94.5	580	4

The method for determining solubility was adapted from the method of Wright (*17*). Twenty grams of skim-milk powder were transferred to a beaker, to which was added 200 cc of nitrogen-free water at 20° C. The beaker was placed in a water bath of 20°, and the contents stirred mechanically for exactly 30 minutes. Fifty cubic centimeters of the mixture were transferred to each of two centrifuge tubes and whirled at 1,850 revolutions per minute for exactly 15 minutes. Three 10-cc samples of the supernatant fluid were removed from one centrifuge tube for moisture determination, while from the second centrifuge tube three 10-cc samples were taken for the determination of nitrogen.

The colors were analyzed with a Keuffel and Esser color analyzer or spectrophotometer. For these analyses the samples were ground in a mortar until all material passed through a 100-mesh sieve. The three characteristics of color—brightness, dominant wave length, and purity—are expressed numerically. As most of these powders were white or very nearly so, it is believed that the figures for brightness are the most significant.

The data of table 1 show that, with a single exception for solids and none for nitrogen, the solubility of the roller-process powders decreased as the severity of the heat treatment increased. The spray-process powders were much more soluble than the roller-process powders, and of the two former the preheated was the less soluble. Also the spray-process powders possessed a greater brightness rating than the roller-process powders, while among the latter, brightness decreased as the intensity of heat processing increased.

In many of the experiments the paired-feeding method (*9*) was used, while for the determination of the biological values of protein the nitrogen-balance method developed in this laboratory (*7*, *10*) was followed.

EXPERIMENTAL RESULTS

THE DIGESTIBILITY OF ENERGY

The apparent digestibility of energy was determined upon two of the roller-process powders, the low-temperature powder and the scorched powder, representing the two extremes of heat treatment. This determination was made upon eight pairs of rats which were being used in a feeding experiment designed to compare the net-energy values of these powders. The results of the net-energy test are presented below. The rations compared contained about 63 percent of the respective powders. The collection periods were of 7 days duration and a feces marker was used.

The average coefficients of apparent digestibility of the energy of these two diets were 90.5 for the low-temperature powder and 89.2 for the scorched powder. In all pairs a higher coefficient was obtained with the low-temperature powder. Such a consistent result would be produced by chance only once in 128 trials, so that its significance is established without further statistical analysis. It may be concluded that the extreme heat employed in preparing the scorched powder depressed the digestibility of its energy in the animal body. Although the average depression for the ration as a whole was 1.375 percent, that of the powder itself, making up only 62.3 percent of the ration, must have been $1.375 \div 0.623 = 2.21$ percent.

THE DIGESTIBILITY OF PROTEIN

The digestibility of the protein in raw liquid skim milk and in the various skim-milk powders was determined from the nitrogen metabolism data secured for the purpose of computing the biological values of these protein mixtures. In these metabolism trials the various samples were compared in turn with the low-temperature (r. p.) powder, the same rats being used in each comparison in groups of 5, 8, or 10. The average coefficients of true digestibility (including due allowance for the metabolic products in the feces) are given in table 2.

TABLE 2.—*Average coefficients of true digestibility of protein for the various experimental skim-milk samples*

Rats (number)	Raw liquid skim milk	Roller-process powders				Spray-process powders	
		Low temperature	Choice commercial	Slightly scorched	Scorched	Not preheated	Preheated
8	94.8	92.7					95.3
5		90.6	93.4			92.0	
5		[1] 91.7		88.8		92.0	
5		[1] 92.1			81.4		

[1] Average of 10 determinations, 2 on each rat.

If digestion coefficients obtained for any two samples on the same rat are considered as paired observations, then Student's (*15*) method for the statistical analysis of small groups of such data may be applied. Such an analysis of the individual data shows that the proteins of liquid skim milk are significantly more digestible than the proteins of

the low temperature (r. p.) powder, and that the latter proteins are significantly better digested than those of the slightly scorched (r. p.) and scorched (r. p.) powders. All other differences are statistically insignificant.

These results are only in partial agreement with those of Nevens and Shaw (*13*), who were able to demonstrate a significantly greater apparent digestibility of the proteins of fresh whole milk than of the proteins of whole-milk powder prepared by either the spray process or the roller process. In these studies the sole diet of the experimental rats consisted of the milk products under test, while in the investigations reported in this article the experimental diets contained less than 25 percent of milk solids. The former dietaries would be more favorable to the detection of differences in the digestibility of milk products than would the latter.

THE NET-ENERGY VALUE

The two skim-milk powders representing the two extremes in heat treatment; i. e., the low-temperature and the scorched roller-process powders, were compared with reference to their availability as sources of energy in animal nutrition. They were incorporated into rations containing other sources of protein, minerals, and vitamins in presumably adequate amounts, so that the milk solids need supply only energy-yielding nutrients. The composition of the rations is given in table 3. The water was added to diet 2 in order to equalize the moisture content of the two powders (table 1).

TABLE 3.—*Composition of the diets used in the comparison of the net-energy values of low-temperature (r. p.) skim-milk powder and scorched (r. p.) powder*

Constituent	Diet 1	Diet 2	Constituent	Diet 1	Diet 2
	Percent	*Percent*		*Percent*	*Percent*
Low-temperature (r. p.) powder	63.50	----	Dried yeast [2]	5.00	5.00
Scorched (r. p.) powder	----	62.34	Cod-liver oil	2.00	2.00
Casein	25.00	25.00	Water	----	1.16
Modified Osborne and Mendel salts [1]	4.50	4.50			

[1] See Wesson (*16*). [2] Obtained from the Northwestern Yeast Co.

The rations were compared with respect to growth-promoting power by means of the paired feeding method, using eight pairs of rats. The growth results are assembled in table 4.

TABLE 4.—*Comparison of the net-energy value of low-temperature powder (r. p.), diet 1, and scorched powder (r. p.), diet 2, as determined by the paired-feeding method during a feeding period of 56 days*

Pair no. and powder used in diet	Total food	Initial weight	Final weight	Gain	Pair no. and powder used in diet	Total food	Initial weight	Final weight	Gain
Pair 1, males:					Pair 5, males:				
Low-temperature powder	*Grams* 527	*Grams* 49	*Grams* 155	*Grams* 106	Low-temperature powder	*Grams* 446	*Grams* 40	*Grams* 131	*Grams* 91
Scorched powder	527	51	145	94	Scorched powder	446	40	113	73
Pair 2, males:					Pair 6, females:				
Low-temperature powder	557	50	160	110	Low-temperature powder	469	46	122	76
Scorched powder	557	47	157	110	Scorched powder	469	46	126	80
Pair 3, females:					Pair 7, females:				
Low-temperature powder	527	48	134	86	Low-temperature powder	423	46	109	63
Scorched powder	527	50	144	94	Scorched powder	423	53	109	56
Pair 4, females:					Pair 8, males:				
Low-temperature powder	479	41	124	83	Low-temperature powder	499	52	143	91
Scorched powder	479	40	123	83	Scorched powder	499	51	151	100

After the feeding period was well under way, it appeared that both diets were deficient in vitamin B. In order to maintain appetite, each rat was given daily by mouth from 3 to 6 drops of tikitiki extract, pair mates being treated exactly alike.

The gains in weight of pair mates reveal no superiority of diet 1 over diet 2. In 3 pairs the rat on diet 1 gained the faster, in 3 pairs the reverse was true, while in 2 pairs the gains were equal.

Since it was possible that a difference in net-energy value between diets 1 and 2 produced a difference in the energy balance rather than in the rate of gain of the rats, the carcasses of four pairs, nos. 1, 3, 5, and 8, were analyzed for gross-energy content, using the Parr oxygen bomb calorimeter. Their contents of nitrogen were also determined. The results are summarized in table 5.

TABLE 5.—*Nitrogen and energy contents of the carcasses of 4 pairs of rats reared on diets 1 and 2* [1]

Pair no.	Dry skim-milk powder in diet	Empty body weight	Nitrogen content		Energy content	
					Per gram	Total
		Grams	*Percent*	*Grams*	*Calories*	*Calories*
1	Low temperature	150	2.86	4.29	1.32	198
	Scorched	139	2.77	3.85	1.32	183
3	Low temperature	129	2.94	3.79	1.32	170
	Scorched	138	2.44	3.37	1.32	182
5	Low temperature	127	2.79	3.54	1.33	169
	Scorched	108	2.86	3.09	1.32	143
8	Low temperature	138	3.04	4.20	1.31	181
	Scorched	145	2.96	4.29	1.31	190

[1] Diet 1 contained the low temperature and diet 2 the scorched powder.

The energy content per gram of empty carcass was remarkably constant for all rats examined, and the total energy content of pair mates showed no consistent differences induced by the two diets. In 2 pairs the rat subsisting on diet 1 stored the greater amount of energy in its body, while in the remaining 2 pairs the reverse was true.

The nitrogen content of the carcass of the rat receiving the low-temperature powder (diet 1) was higher, both on the percentage and the absolute basis, in 2 of the 4 pairs than that of the rat receiving the scorched powder, but these differences are not sufficiently large or consistent or numerous to indicate with any great degree of certainty, according to statistical analysis, that they were the result of the difference in diet consumed rather than the result of chance.

The digestibility coefficients above discussed indicate clearly that the energy of the scorched skim-milk powder was somewhat less digestible than that of the low-temperature powder. The failure to demonstrate a difference in the net-energy values of the two powders by the growth experiment may be the result of (1) a greater degree of activity of the rats on the diet containing the low-temperature powder, (2) a greater specific dynamic effect of this diet or (3) a biological error in the growth experiment sufficient to obscure the effect of the greater digestibility of the low-temperature powder.

In all probability any difference in net-energy value between the low-temperature skim-milk powder and the scorched powder is inconsiderable. It may be concluded further that powders subjected to

heat insufficient in intensity to produce more than a barely perceptible scorching are for all practical purposes equally valuable as sources of energy in the animal body.

BIOLOGICAL VALUE OF THE PROTEINS

The general plan of experimental procedure in determining the biological value of the proteins was that developed by Mitchell (*7*, *10*), except that in these metabolism studies feces markers [4] were employed. The compositions of the various diets are presented in table 6. In order to improve the consumption of the experimental diets, Harris yeast vitamin powder was fed. This vitamin concentrate was fed in some instances as part of the diet, in others as part of the diet supplemented by additional amounts weighed into individual feed dishes, and later by feeding all of it as an addition to the mixed diet, which accounts for the presence of more than 1 percentage of this constituent in a given diet. As the amount of vitamin powder was changed in the diet, a corresponding and equal change was made in the amount of starch.

TABLE 6.—*Composition of the diets used in studying the relative biological values of the proteins of raw liquid skim milk and the various dry skim-milk powders*

Constituents	Standardizing diet	Raw liquid skim milk	Low-temperature powder (r. p.)	Choice commercial powder (r. p.)	Slightly scorched powder (r. p.)	Scorched powder (r. p.)	Not preheated powder (s. p.)	Preheated powder (s. p.)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dried fat-free whole egg	5.47 or 5.35							
Raw liquid skim milk		[1] 22.55						
Low-temperature powder (r. p.)			23.66					
Choice commercial powder (r. p.)				23.27				
Slightly scorched powder (r. p.)					23.66			
Scorched powder (r. p.)						23.06		
Not preheated powder (s. p.)							23.70	
Preheated powder (s. p.)								23.27
Modified Osborne-Mendel salts	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Filtered butterfat	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Cod-liver oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Sucrose	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
NaCl	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Harris yeast vitamin powder	.5 or .0		.3 or .5 or .0	.5	.5	.5		
Starch	66.53 or 67.15	49.95	48.54 or 48.34 or 48.84	48.73	48.34	48.94	48.80	49.20
Pigment	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

[1] Dry substance.

The calculations of the biological values of the proteins of the various milk samples was made from the nitrogen metabolism data according to the usual procedure followed in this laboratory (*10*); hence it seems unnecessary to give them in detail here. The biological

[4] On the first day of each experimental period the diet fed contained 2 percent of ferric oxide, which marked the feces red. On the remaining days of the experimental period, in most cases of 7 days' duration, an equal percentage of barium sulphate was included in the diet. This produced a progressively lighter color in the feces. On the first day following an experimental period, or the first day of a transition period, which was always 4 days in length, the diet contained 2 percent of chromic oxide to mark the feces green.

value is the percentage of the absorbed nitrogen that is retained for both maintenance and growth under the standard conditions adopted for the determination. The estimate of the absorbed nitrogen makes due allowance for the metabolic nitrogen excreted in the feces, the ratio between metabolic fecal nitrogen and dry-matter intake being measured in the standardizing periods. The estimate of the total storage of nitrogen in the body makes due allowance for the endogenous nitrogen excreted in the urine, the ratio between endogenous nitrogen and body weight being also determined in the standardizing periods.

The various milk samples were compared in turn with respect to the biological value of their protein components with the low-temperature (r. p.) powder, groups of 5, 8, or 10 rats being used in each comparison. The average biological values for each group of rats and for each milk sample are assembled in table 7.

TABLE 7.—*Average biological values of protein for the various experimental skim-milk samples*

Rats (number)	Raw liquid skim milk	Roller-process powders				Spray-process powders	
		Low temperature	Choice commercial	Slightly scorched	Scorched	Not preheated	Preheated
8	89.8	89.1					82.3
5		89.6	81.6			87.2	
5		[1] 89.3		68.0		88.6	
8		[1] 87.9			69.8		

[1] Average of 10 determinations, 2 on each rat.

The average biological value of 89.8 for the proteins of liquid skim milk may be compared with the average of 95 obtained by Shiftan (*14*) with two pigs fed a ration containing about 12 percent of protein. The average of 82.3 for preheated skim-milk powder (s. p.) is very close to the value of 85 obtained in this laboratory in two investigations (*8*, *10*) with milk powders of this description, and also with the value of 86 reported by Boas Fixsen and Jackson (*2*), relating to a whole-milk powder manufactured by the roller process.

The biological values (single or average) obtained for different samples on the same rats have been considered as paired observations, and the differences between them have been subjected to statistical analysis according to the method of Student (*15*). The statistical results are summarized in table 8. From these analyses it may be concluded that the nutritive value of the digestible proteins of fresh skim milk is not depressed by drying by the roller process at the lowest feasible temperature, nor by drying by the spray process provided the preheating is dispensed with. However, if drying is accomplished by the prevailing commercial processes, yielding products represented by the choice commercial (r. p.) sample and the preheated (s. p.) sample, a definite lowering of nutritive value occurs, equal to about 8 percent. If drying by the roller process is so poorly controlled that even slight scorching occurs, then a much greater decrease in nutritive value is effected, amounting to more than 20 percent.

TABLE 8.—*Analysis according to Student's method of the differences in biological values between the proteins of dry skim-milk powders and of liquid skim milk*

Statistical item	Raw liquid skim milk *v.* low-temperature powder (r. p.)	Low-temperature powder (r. p.) *v.* not preheated powder (s. p.)	Raw liquid skim milk *v.* preheated powder (s. p.)	Low-temperature powder (r. p.) *v.* preheated powder (s. p.)	Low-temperature powder (r. p.) *v.* choice commercial powder (r. p.)	Low-temperature powder (r. p.) *v.* slightly scorched powder (r. p.)	Low-temperature powder (r. p.) *v.* scorched powder (r. p.)
Mean of differences, M	0.625	1.7	7.5	6.875	7.8	21.6	18.2
Standard deviation of differences, s	4.22	4.24	3.54	3.14	1.6	2.32	3.25
Probability, P	.35	.13	.0004	.0003	<.0019	<.0019	.0019

TOTAL EFFECT OF DRYING UPON THE NUTRITIVE VALUE OF SKIM-MILK PROTEINS

The relative protein values of the various milk samples tested, which take account of differences in digestibility of protein as well as differences in biological value, have been computed and the results are presented in table 9. These values, listed in the last column of the table, are expressed in percentages, the value of raw liquid skim milk being taken as 100. From these values it appears that skim milk may be dried with a loss in protein value of only 5 percent or less, but that by the ordinary processes of commercial drying losses of 9 to 11 percent occur, and if there is perceptible scorching through careless management the loss may be 30 percent.

TABLE 9.—*Relative protein values of the different milk samples tested* [1]

Sample	Average true digestibility of protein	Average biological value	Relative protein value	Sample	Average true digestibility of protein	Average biological value	Relative protein value
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
Raw liquid skim milk	95	90	100	Spray-process powders:			
Roller-process powders:				Not preheated	92	88	95
Low temperature	91	89	95	Preheated	95	82	91
Choice commercial	93	82	89				
Slightly scorched	80	68	71				
Scorched	81	70	66				

[1] Expressed as percentages with the relative protein value for raw liquid skim milk taken as 100.

Thus, the data obtained demonstrate a point of tremendous practical importance, namely, that the proteins of milk are very sensitive to heat with respect to their value in nutrition, and that considerable losses in protein efficiency may be incurred unless the time of exposure to heat and the intensity of the heat are carefully controlled. In this respect the requirements for the highest engineering efficiency may conflict with the requirements for the highest quality of product, and the dry-milk industry must decide which requirements are paramount.

SEAT OF INJURY TO THE PROTEINS OF MILK PRODUCED BY DRYING IN TERMS OF THE CONSTITUENT AMINO ACIDS

Having established the existence of definite deterioration of skim-milk proteins during commercial drying processes, as well as the

extent of such deterioration, the next step in the investigation was to discover, if possible, which of the constituent amino acids was involved in the successive decreases in nutritive value as the severity of the drying process was intensified. Such information would be afforded by a study of the amino acids limiting the nutritive values of the various samples of skim-milk powders.

In this study the paired-feeding method was used to compare the growth-promoting powers of rations containing the various milk powders as the sole source of protein and the same rations with small supplements (0.3 percent) of selected amino acids. The rations all contained enough of the milk powders (23 to 27 percent) to supply approximately 9 percent of protein, 4.5 or 5.0 percent of the Wesson (*16*) modification of the Osborne and Mendel salt mixture, 1 percent of NaCl, 10 or 12 percent of sucrose, 2 percent of cod-liver oil, 8 percent of butterfat, 10 or 12 percent of lard, and enough starch to make 100 percent. Vitamins B and G were supplied extra as Harris yeast vitamin powder.

The deficiency of the low-temperature (r. p.) powder in cystine was readily demonstrated in a 14-day feeding test with eight pairs of rats. In this short period the rat in each pair given the cystine supplement gained more in weight than its pair mate on the unsupplemented diet on the same amount of food and attained a greater body length. The average excess gain by the test rats was 7.37 g, the standard deviation of excess gains was 2.75 g, and the probability that fortuitous factors would have produced so consistent an outcome is only 0.0001, according to Student's (*15*) probability tables. The average difference in body length was 5.50 mm, the standard deviation of differences 3.94 mm, and the probability of a chance outcome only 0.0038.

That preheated skim-milk (s. p.) powder is also deficient in cystine had been previously demonstrated by Mitchell and Beadles (*9*), the skim-milk powder used being of this description.

The evidence thus far adduced indicates that the initial drop of about 9 to 11 percent in the nutritive value of skim-milk proteins during drying by prevailing commercial methods must be the result of a destruction of cystine, since cystine is still the amino acid limiting the biological value of commercial skim-milk powders. However, the growth-promoting value of the slightly scorched (r. p.) powder was not improved by a cystine supplement. Eight pairs of rats were used in this test, and at the end of 2 weeks the gains of pair mates were very nearly the same, being exactly equal in 2 pairs, only 1 g apart in 4 pairs, 2 g apart in 1 pair, and 5 g apart in the remaining pair. The rats receiving cystine supplements gained more than their control mates on the same amount of food in only 2 pairs. At this point in the feeding experiment the control rats, previously consuming the unsupplemented diet, were given a supplement of lysine dihydrochloride equal to 0.3 percent of the basal diet. The other rats in each pair continued on the basal diet plus cystine.

Three weeks after this change in plan was put into effect, the rats receiving the lysine supplement had exceeded in gain their pair mates receiving the cystine supplement in all of the 8 pairs and had also attained to greater body lengths in all pairs. The average difference in gain between pair mates was 6.37 g, the standard deviation of differences 2.19 g, and the probability that a purely random com-

bination of factors common to both pair mates would produce as consistent an outcome as this is negligible, amounting to less than 0.0001. The average difference in body length (from nose to root of tail) is 5.25 mm, the standard deviation of differences, 3.23 mm, and the desired probability only 0.0018, again entirely negligible. Body length measurements, somewhat more surely than body weight measurements, are reliable criteria of growth, and in this case demonstrate beyond question that a lysine supplement to the proteins contained in the slightly scorched skim-milk powder increased their growth-promoting value. The complete data of this test will be found in table 10.

TABLE 10.—*Effects of supplements of cystine alone for 2 weeks and of cystine and lysine for 3 weeks on the growth-promoting value of slightly scorched skim-milk powder (r. p.)*

Results of the first 2 weeks of feeding					Results of the last 3 weeks of feeding					
Pair no. and diet or supplement	Total food	Initial weight	Final weight	Gain	Pair no. and supplement	Total food	Initial weight	Final weight	Gain	Body length
	g	*g*	*g*	*g*		*g*	*g*	*g*	*g*	*mm*
Pair 1, females:					Pair 1, females:					
Basal only	109	63	87	24	Lysine	164	87	118	31	182
Cystine	109	58	82	24	Cystine	164	82	106	24	173
Pair 2, females:					Pair 2, females:					
Basal only	97	56	74	18	Lysine	180	74	111	37	175
Cystine	97	56	73	17	Cystine	178	73	108	35	169
Pair 3, females:					Pair 3, females:					
Basal only	77	48	62	14	Lysine	142	62	93	31	163
Cystine	77	48	61	13	Cystine	144	61	88	27	160
Pair 4, females:					Pair 4, females:					
Basal only	85	46	62	16	Lysine	148	62	91	29	163
Cystine	85	47	64	17	Cystine	148	64	86	22	159
Pair 5, females:					Pair 5, females:					
Basal only	103	51	74	23	Lysine	186	74	114	40	173
Cystine	103	49	67	18	Cystine	186	67	99	32	167
Pair 6, females:					Pair 6, females:					
Basal only	102	53	70	17	Lysine	170	70	103	33	168
Cystine	102	54	69	15	Cystine	170	69	93	24	166
Pair 7, females:					Pair 7, females:					
Basal only	108	53	75	22	Lysine	184	75	112	37	176
Cystine	108	51	73	22	Cystine	184	73	102	29	165
Pair 8, females:					Pair 8, females:					
Basal only	96	47	67	20	Lysine	204	67	115	48	172
Cystine	96	48	69	21	Cystine	204	69	111	42	171

The sample of scorched skim-milk powder (r. p.), by similar paired-feeding tests, was found not to be improved in growth-promoting properties by supplements of cystine, histidine, or tryptophane. However, when supplemented by lysine, this sample, like the slightly scorched sample, exhibited a clear improvement in its power to promote growth. Only four pairs of rats were used in this test, with the results summarized in table 11. In each pair, the rat receiving the lysine supplement gained the faster and attained the greater body length. In spite of the fact that only four pairs of rats were used, the results are quite highly significant. Thus, the average difference in gain between pair mates was 7.75 g, the standard deviation of differences 4.09 g, and the probability of a fortuitous outcome, only 0.026. The average difference in body length was 5.75 mm, the standard deviation of differences 2.86 mm, and the desired probability only 0.020.

TABLE 11.—*Effect of a lysine supplement on the growth-promoting value of the proteins of scorched skim-milk powder (r. p.)*

Pair no. and diet or supplement	Total food	Initial weight	Final weight	Gain	Body length
	Grams	*Grams*	*Grams*	*Grams*	*Millimeters*
Pair 1, females:					
Basal only	310	59	89	30	161
Lysine	307	57	88	31	163
Pair 2, females:					
Basal only	359	61	94	33	163
Lysine	359	61	105	44	172
Pair 3, females:					
Basal only	278	49	72	23	153
Lysine	278	49	80	31	157
Pair 4, females:					
Basal only	285	55	72	17	154
Lysine	285	55	83	28	162

From this series of paired-feeding experiments it appears that the initial stages of the destruction of milk proteins by heat, occurring during the drying according to prevailing commercial processes, involves, and is the direct result of, a destruction of cystine, but that the later stages, from the initiation of perceptible scorching to the production of a thoroughly scorched product, involve a more rapid destruction of lysine than of cystine. This is a finding of considerable practical importance, since the value of milk proteins in supplementing the proteins of the cereal grains is the result of the deficiency of the latter in lysine and of the presence in the former of abundant proportions of this amino acid. Hence, scorched skim-milk powders, merchandisable only for animal feeding, would not possess this supplementing capacity.

CONCLUSIONS

The proteins of milk are very sensitive to the intensities and durations of heat treatment employed in commercial drying. However, it is possible to dry skim milk with commercial equipment without appreciably affecting its energy value or the nutritive value of its proteins. In the preparation of choice commercial roller-process powders, or of preheated spray-process powders, the biological value of the protein is lowered about 8 percent (from 90 to 82), although its digestibility is not appreciably affected. If preheating is omitted in the spray-drying process this reduction in nutritive value of the milk proteins does not occur. Since cystine is the amino acid limiting the biological value of the proteins of choice commercial roller-process powder and preheated spray-process powder, as well as of the original milk, it may be concluded that this initial decline in biological value of milk proteins is due to a partial destruction of cystine.

As the temperature of drying in the roller process is increased until perceptible scorching occurs the biological value of the milk proteins is rapidly lowered from 82 to 70 or less. The scorched products thus obtained are no longer benefited by cystine additions, but they do respond to lysine additions in increased nutritive value of their proteins. Hence the rapid change in milk proteins at the scorching point (or earlier) is primarily a result of the destruction of lysine. Such products, therefore, are of no value as supplements to the proteins of cereal grains. The digestibility of the milk proteins is also lowered at the scorching point in the roller-drying process, the extent

of lowering increasing more rapidly with the degree of scorching than the reduction in biological value.

However, even with extreme scorching, the net-energy value appears to be but slightly (if at all) affected. Apparently the protein disintegration occasioned by scorching conditions does not impair appreciably the value of the protein as a source of energy to the body.

The solubility of the total solids and the nitrogen of dry skim-milk powders is greater for spray-process than for roller-process powders and decreases in the latter with the intensity of the drying conditions. The spray-process powders gave a higher percentage of brightness than the roller-process powders when colors were analyzed, and in general brightness decreased as the heat increased. These solubility differences and color differences, however, are not reliable criteria of the changes occurring in the nutritive value of the proteins.

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EVALUATION OF LENGTH MEASUREMENT IN AN EXPERIMENT WITH APPLE TREES[1]

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INTRODUCTION

Adequate measurement of experimentally induced differences in fruit trees is rarely attained. Yields obviously constitute the results of greatest ultimate interest, but unsupported yield records usually tell little about the precise mechanism by which the yields were secured and consequently have limited value as indicators of the general applicability of the results. Wood-growth measurements may represent in some degree what the tree has done, but leaf surface may be a better indicator of what it is about to do. Quantitative measurements of any sort, whether of wood growth, spur number, or leaf surface, present incomplete pictures since they do not well represent distribution, and the experienced fruit grower or investigator may have some justification for his belief that his eyes will tell him more about a tree than any amount of data will reveal.

Quantitative measurements, nevertheless, are obviously necessary, and the realization of their imperfections should only stimulate study leading to their improvement. Though no one index figure may ever be attained that will express all the performance and potentiality of a tree, various standards may be used to represent it at various stages, and each should be improved as far as possible.

The best measure of accomplished vegetative growth is generally considered to be the weight of dry matter produced. Since complete determination of this manifestly terminates an experiment, some sort of measurements of size seems to be the best means of gaging growth that is possible in many cases. Trunk girth measurements are of undoubted value, particularly in trees not yet bearing, but have inherent limitations in the relatively great importance of small errors in measurements as well as difficulties of execution due to trunk excentricity, sunscald, wounds, proximity of scaffold limbs, etc. Consequently length has been widely used.

In actual application, however, length measurement may present complications. On large trees enough analogous shoots may be chosen to yield a constant average, i. e., one that is not modified by duplicate sampling. Any comparison of trees made by this standard is predicated on the assumption, usually not established, that the number of comparable shoots is identical. For a short time after experimental differences, as for example by soil treatments, have been produced, this assumption is justifiable; in pruning experiments of any duration and after several years of soil treatments it is questionable. The possible fallaciousness of mean growth is illustrated by some measurements on young apple trees, where all growths of 10 cm, or over, in 1934 were recorded. Two trees had identical means, 50.2 cm, but on one this was an average of 20 shoots, while, on the other, 29 shoots

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contributed to the average; the respective total growths (for 1934) were 1,004 and 1,456 cm. This being true, its converse may be anticipated; the writers' records show another pair of trees with total growths of 833 and 836 cm, respectively, representing the total lengths of 18 shoots in the former and of 40 in the latter, with consequent respective averages of 46.3 and 20.9 cm.

Total length is an unreliable measure of growth, if weight is a good measure. Weights of 1-year shoots are not directly proportional to their lengths, where differences of much consequence exist. The mean fresh weights per linear centimeter in dormant 1-year shoots of Red Canada (Steele Red) (samples of 25 to 30 specimens each) varied with length, as follows: 25 cm, 0.09 g; 35 cm, 0.12 g; 45 cm, 0.14 g; 55 cm, 0.17 g; 65 cm, 0.21 g. In the pair of trees just mentioned, having 833 and 836 cm, respectively, of total growth in 1934, the actual weight of new wood in the former was 34 percent greater than in the latter. In the pair of trees mentioned first, the one with 1,456 cm total growth had not produced merely 45 percent more weight than the one with 1,004 cm—it had virtually doubled it. Even more surprisingly, simple total-length measurements can make the smaller appear to be the larger, as will be shown presently.

Greater accuracy in application of length measurements might be expected from the use of a scale which made allowance for an increasing weight per unit of length as length increases. The applicability of any graduated scale of this sort obviously depends in considerable degree on the variability of the material. An account of the construction of two graduated scales and their application to specimens of known weight and to trees under experimental treatment follows.

MATERIAL AND METHOD

The material used was taken from Red Canada apple trees in their second and third seasons in the orchard of the Michigan Agricultural Experiment Station at East Lansing. These trees are pruned only to secure proper spacing of scaffold limbs and receive little or no tipping back; consequently the prunings contain the full season's growth of the shoots removed. While these trees still appeared completely dormant in March 1935, the regular pruning was done, and the prunings from each tree were tied in a bundle and tagged. The total weight and the weight of the 1934 wood were recorded in the laboratory for each bundle, and each shoot was measured. All shoots of 1934 growth were then assembled, sorted into length classes (10-cm intervals), the weight of each class was determined, and the component shoots were counted. The means thus secured constitute the scale here called graduated scale A.

The values on this scale, when graphed, fitted rather well the equation—

$$X^2=3.1\ Y$$

where X equals the length in centimeters and Y the weight in grams. Individual weights and lengths of 1,428 of these shoots showed a straight-line correlation coefficient of 0.32 ± 0.016. Computed from deviations from the values in the equation $X^2=3.1\ Y$, the index of correlation [2] was 0.93, indicating the closer fit of the parabola.

[2] $\rho_{yY}=\sqrt{1-\frac{S_y^2}{\sigma_y^2}}$.

Since the weight values assigned in scale A change abruptly and considerably (table 1) another scale, called B, assigning values to each centimeter increase in length, was constructed by smoothing 1,428 records of individual weights and lengths. These, when tabulated for each centimeter of length, beginning with 21 cm, exhibited rather striking uniformity. For example, the probable error of weights of shoots 25 cm long was 3.11 percent of the mean; for shoots 35 cm long it was 2.38 percent; and for 65 cm, 1.89 percent. The values used in the scale were moving averages, smoothed slightly; for values below 21 cm scale A was used.

To bring simple length measurements into a denomination comparable with the graduated scales, the mean weight per centimeter of all shoots studied, 0.17022 g, was shortened to 0.17, and all lengths were multiplied by this factor; this constitutes the simple scale.

After length and fresh weight had been determined, all wood was dried to constant weight in an oven at 100° C. The dry weights thus determined bore a uniform relationship to the fresh weights and showed nothing new. The studies here reported are, therefore, confined to fresh-weight figures.

To try the accuracy of the three scales, weights of prunings from each tree were then calculated by each scale and compared with the actual weights previously recorded (table 2).

TABLE 1.—*Values used in computing weight of shoot from length by scales A and B and by simple weight*

Graduated scale A		Graduated scale B		Simple weight
Length	Weight	Length	Weight	
Centimeter	*Grams*	*Centimeter*	*Grams*	*Grams*
0–10	0.4			
11–20	1.3	20	1.3	3.4
21–30	2.3	25	2.2	4.3
		30	3.0	5.1
31–40	4.2	35	4.2	6.0
		40	5.3	6.8
41–50	6.6	45	6.3	7.7
		50	7.6	8.5
51–60	9.5	55	9.3	9.4
		60	11.1	10.2
61–70	13.0	65	12.9	11.1
		70	15.1	11.9
71–80	18.0	75	18.1	12.8
		80	21.1	13.6
81–90	23.5	85	24.8	14.5
		90	28.3	15.3
91–100	32.4	95	31.4	16.2
		100	34.6	17.0
100–110	40.5	105	37.4	17.9
		110	----------	18.7

Individual records from four pairs of trees are shown in table 2 as illustrative of the varying significance of length measurements. The prunings representing trees A and B, judged by simple length measurements, were virtually identical; in actual weight there was a difference of nearly 46 percent. Between the prunings from trees C and D there was a length difference of 41 percent, while the weight difference was less than 1 percent. Tree E's prunings were more than 14 percent greater in length, and 14 percent less in weight, than those of

tree F. Similarly the shoots removed from tree G were 14 percent longer than those of tree H, but weighed 31 percent less. Had these shoots remained on the trees and been compared simply on the basis of shoot length, some very real difference would have been obscured. The graduated scales gave greater accuracy than length measurements in seven of the eight comparisons.

In some cases the graduated scales give weight indications at considerable variance from the actual. In a large number of determinations, however, the results secured by these methods approximate the actuality rather well. In the 155 cases for which data are available, scale A gave closer approximation than the simple scale in 69.7 percent of the cases, and scale B was more accurate than the simple scale in 69.4 percent. Deviations as calculated from the actual weights by the graduated scales were about half those secured by the simple scale, as shown in table 3.

TABLE 2.—*Effect of length of shoot on weight of wood produced*

Item	Data for tree—							
	A	B	C	D	E	F	G	H
Total growth.....centimeter..	507	503	463	328	800	698	647	566
Shoots of indicated length in centimeters:								
10-20.....number..	5	.....	2	.....	9	.....	1	.....
21-30.....do....	2	.....	3	.....	1	.....	1	.....
31-40.....do....	7	1	7	3	4	.....	4	1
41-50.....do....	3	1	2	3	5	2	5	.....
51-60.....do....	.....	3	.....	.....	2	3	3	4
61-70.....do....	.....	4	.....	1	.....	3	1	2
71-80.....do....	.....	.....	.....	.....	1	1	.....	1
81-90.....do....	.....	.....	.....	.....	1	2	.....	.....
91-100.....do....	.....	.....	.....	.....	.....	.....	.....	1
Total shoots.....	17	9	14	7	23	11	15	9
Calculated weight by—								
Scale A.....grams..	59.7	91.7	52.7	45.4	123.2	145.7	95.1	118.6
Scale B.....do....	60.6	88.1	53.8	50.3	130.4	151.0	95.0	121.5
Simple.....do....	86.2	85.5	78.7	55.8	136.0	118.7	110.0	96.2
Actual weight.....do....	63.8	93.0	52.5	52.0	124.1	144.6	96.0	139.6

TABLE 3.—*Deviation from the actual of weights calculated from length measurement by use of graduated scales and of average weight*

Item	Scale A		Scale B		Length (centimeters)×0.17	
	Cases	Mean deviation	Cases	Mean deviation	Cases	Mean deviation
Deviations:	*Number*	*Grams*	*Number*	*Grams*	*Number*	*Grams*
Above actual	72	3.75	57	4.23	103	8.45
Below actual	83	5.75	96	5.98	51	15.82
Total	155	.....	[1] 153	.....	[1] 154	.....
Error.....percent..	.....	4.8	.....	5.3	.....	10.8
Percentage error	.....	7.7	.....	8.4	.....	17.2

[1] No deviation in 2 cases and in 1 case, respectively.

For the entire group the several coefficients of variability of prunings, tree by tree, were: Length, 0.63; actual weight, 0.74; scale A, 0.71; scale B, 0.74.

Both graduated scales underestimate weight more frequently than they overestimate it, and the margin of error is greater in underestimation than in overestimation. Comparison of the 9 cases of greatest overestimation by scale A with the 10 cases of greatest underestimation by this method shows that overestimation occurred predominantly in cases involving longer growths, underestimation in cases in which shorter growths were more numerous. In the cases of greatest overestimation the percentage of shoots measuring under 31 cm was 12.8; in those of greatest underestimation it was 25.5; in overestimation the percentage of shoots over 70 cm was 20.2, while in underestimation it was 7.8.

Whether the pruning, which was done solely to secure proper spacing of scaffold limbs, removed samples which were representative, and whether the differences found in prunings would be paralleled if all growths, pruned and unpruned, were considered, is answered affirmatively by the data (table 4). The overturnings in relative standings here are similar to those shown in the prunings.

TABLE 4.—*Relative rankings of trees on bases of measured length and calculated weight of all 1934 growths over 9 cm in length*

Tree	Length growth	Calculated weight	Relative rank for—		Frequency distribution of shoots of length indicated									
			Length	Weight	10 cm	11–20 cm	21–30 cm	31–40 cm	41–50 cm	51–60 cm	61–70 cm	71–80 cm	81–90 cm	91–100 cm	Total
	Centimeters	*Grams*													*Number*
A	882	99	1	12	2	25	7	3	3	----	----	1	----	----	41
B	878	146	2	2	1	5	5	2	1	3	5	1	----	----	23
C	876	140	3	3	----	3	----	1	7	6	2	----	----	----	19
D	870	104	4	10	1	18	7	4	4	2	----	----	----	----	36
E	869	117	5	7	2	6	1	9	6	3	----	----	----	----	27
F	843	135	6	6	----	1	1	4	5	4	2	----	----	----	17
G	839	69	7	13	8	41	5	----	----	----	----	----	----	----	54
H	838	112	8	9	----	3	8	5	1	5	1	----	----	----	23
I	837	102	9	11	4	20	11	1	2	1	----	1	----	----	40
J	834	137	10	5	----	3	----	1	4	8	2	----	----	----	18
K	823	116	11	8	----	1	4	8	4	2	2	----	----	----	21
L	821	162	12	1	----	1	1	1	2	4	4	1	----	1	15
M	803	138	13	4	----	----	----	2	2	9	1	1	----	----	15

Finally, the question must be considered whether compensating error may not, in comparisons involving fair numbers of trees, offset the differences appearing in comparisons involving a few trees. In some cases it plainly does offset these differences. For example, all trees (27), making 1934 growths between 600 and 699 cm and all (17) making growths between 800 and 899 cm were compared. In average length the latter group exceeded the former by 30.3 percent; in the calculated weight the difference was 31.6 percent in the same direction. Likewise, a lot of 24 trees on one interstock, in their second year in the orchard, exceeded a lot of 44 trees worked directly on seedlings by 30 percent in length growth and by 26.5 percent in weight; in neither case was the difference over three times the probable error of the difference.

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On the other hand, rather important differences appear in another group, a year older, in which the magnitude of relationships indicated by the two standards shifts (table 5). There is no change of consequence in ranking, but the change in degree of difference is considerable. Lot A's superiority over lot D is increased, while lot B's margin over lot D, of doubtful statistical significance on the basis of length measurements, is further reduced by reference to the weight basis.

TABLE 5.—*Comparison of 1934 growth of lots of Red Canada apple trees on bases of observed length and of calculated weight*

Lot	Trees	Length		Weight		Percentage of lot D	
		Mean per tree	Probable error	Mean per tree	Probable error	Length	Weight
	Number	*Centimeters*	*Centimeters*	*Grams*	*Grams*		
A	19	1,373	105.5	223	18.6	161	169
B	17	1,032	97.8	140	16.4	121	107
C	37	957	56.0	139	9.7	112	106
D	19	855	85.2	131	14.4	99	101
E	16	847	85.0	132	14.2		

DISCUSSION

The greater accuracy attained by interpreting growth in terms of weight seems to warrant its consideration in studies involving length measurements, at least in young trees. In some, perhaps in most, cases, little change will be made from results recorded as simple length measurements, but the other cases are likely, for this reason, to be important enough to repay examination of all. In mature trees it seems unlikely to be of great usefulness, since other standards, such as spur production, spur performance, and yield are more valuable. As a means of comparing response of different varieties it seems promising. In interpretation of growths in the nursery it is particularly valuable.

Obviously, this method can be applied only to wood of most recent growth. The weight of 2-year-old wood is affected by the growth made in two seasons; in older wood the weight is a still more complicated product.

The labor involved in use of the graduated scale need not be much greater than that involved in simple length measurements. Once the values are established for each variety and condition, a meter stick can be recalibrated to read directly in grams and the measurements recorded directly, with no increase whatever in labor of recording or calculation.

THE DETERMINATION AND IMPORTANCE OF THE CONDITION OF THE FIRM ALBUMEN IN STUDIES OF EGG-WHITE QUALITY [1]

By A. VAN WAGENEN, *assistant poultry husbandman*, and H. S. WILGUS, JR., *investigator in poultry nutrition, New York (Cornell) Agricultural Experiment Station* [2]

INTRODUCTION

The demand by consumers for eggs of high quality, as shown by market surveys, is reflected in the increasing stress that is being laid on this factor and its measurement in experimental work conducted on poultry. While candling is the basis for establishing the market value of eggs, it is too indefinite to be of more than superficial value in research. Therefore, it has been necessary to study the component parts of the egg. Various investigators have used one or more of the following measurements as criteria of egg quality exclusive of nutritive value: The size of egg; the thickness or strength, porosity, texture, and color of the shell; the color, dimensions, condition, flavor, and odor of the yolk; and the color, relative volume or weight of the various layers of the albumen, and condition of the firm albumen.

The importance of most of these factors has been generally accepted by the majority of investigators. Most workers have used the relative proportions of firm and thin albumen as the sole criterion of albumen quality. The determination of the observed condition, or firmness, of the firm albumen, however, has not received proper recognition. The term "firm albumen" is used throughout this paper to refer to the "true thick albumen" as defined by Sharp (*17*)[3] unless otherwise specifically noted.

It is generally agreed that consumers prefer an egg for table use which holds together well and cooks evenly throughout. The ability of an egg to meet these requirements depends not only upon the quantity of firm albumen but also upon its firmness, body, or condition. There is also the strong probability that this condition of the firm albumen plays an important part in determining the candling properties of the egg, particularly in controlling the apparent mobility, or swing, of the yolk (*10*). On the other hand, Almquist (*1*) has found little correlation between the quantity of firm albumen and the apparent mobility of the yolk. Pennington et al. (*14*) found no relationship between the percentage of thick white and either the candled grade or the yolk index. The reason that no measure of the condition of firm albumen has been generally used in studies of egg quality is undoubtedly that the various physicochemical components which constitute it have not yet been established. However, this is no justification for ignoring an estimation of a factor which plays such

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[3] Reference is made by number (italic) to Literature Cited, p. 1136.

an important part in determining the desirability of eggs from the viewpoint of the consumer and probably of the market man.

The nature of the firm albumen has been subjected to some investigation. It appears unlikely from the work of Almquist and Lorenz (*4*) and of Bronkhorst (*6*) that the difference between firm and thin albumen is entirely chemical. The findings of St. John and Green (*15*) and of Almquist and Lorenz (*3*) strongly suggest that the difference is chiefly a physical one. The latter authors believe that the structure of the firm albumen is due to fibers of ovomucin. Later, McNally (*12*) and Almquist, Givens, and Klose (*2*) showed that the firm albumen contains more ovomucin than the other layers. The findings of the latter authors indicated that photometric methods might be used to detect differences due to the effect, presumably of ovomucin, upon the transmission of light. Such measurements, however, must be correlated with the condition of the firm white as noted by observation. Such an observation necessitates a fixed score capable of reproducible results and of universal application.

A method of scoring the condition of the firm albumen was first used by Sharp (*16*), but he has only recently described and partially illustrated it (*17*). It is the purpose of this paper to illustrate this method more completely, to present further evidence of its importance, and to extend preliminary data presented by the authors (*19*) showing that there is not necessarily a correlation between the condition and the quantity of the firm layer.

EXPERIMENTAL METHODS

In the first part of this study, photographs were taken to illustrate the score for the condition of the firm albumen. The pictures shown in figure 1 were all taken of freshly laid eggs from pullets of the Cornell strain of Single Comb White Leghorns but represent conditions of the firm albumen encountered in both fresh and held eggs. The scores run from 1.0 to 5.0, as was suggested by Sharp, with intervals of 0.5. In using this method, it was soon found that the scores could be determined accurately by intervals of 0.25. Both top and side (silhouette) views were taken of each egg to illustrate the way the albumen stood up and was distributed around and over the yolk. Each egg illustrates the lower limit for its group range; thus the egg marked 1.0 is the poorest egg that may be scored 1.0. The egg illustrating 5.0 is the lowest score, since no thick albumen is discernible.

The two chief factors in this observation are the outline of the firm albumen viewed from the top, and the outline viewed from the side. The firm albumen in an egg with the best score is concentrated around and over the yolk and tends to occupy the least possible area on the plate. From the top view its outline appears to be ovoid. As the score increases, the firm white spreads and the outline tends to become more irregular. That of 2.5 is generally the first to show this markedly. In this case, a definite tendency toward a rupture of the firm albumen is noticeable. The firm layer of an egg with the score of 3.0 is usually about to rupture, or has just ruptured, allowing the inner thin albumen to mix with the outer thin albumen. From the side view, the outline shows no distinct break in sweep between the yolk and the firm albumen in the best egg. As the score increases, the firm white flattens and spreads out and the angle between the yolk

and albumen appears and becomes sharper until it approaches 90°. This occurs at about the time of rupture of the firm-albumen sac.

The score of the observed condition of the firm albumen considers the apparent thick white described by Sharp (*17*). This includes both the true thick and the inner thin albumen up to the point of rupture of the thick-albumen sac. From this point (score 3.0) on, only the true thick albumen, chiefly structural albumen, is scored since the inner thin layer has escaped.

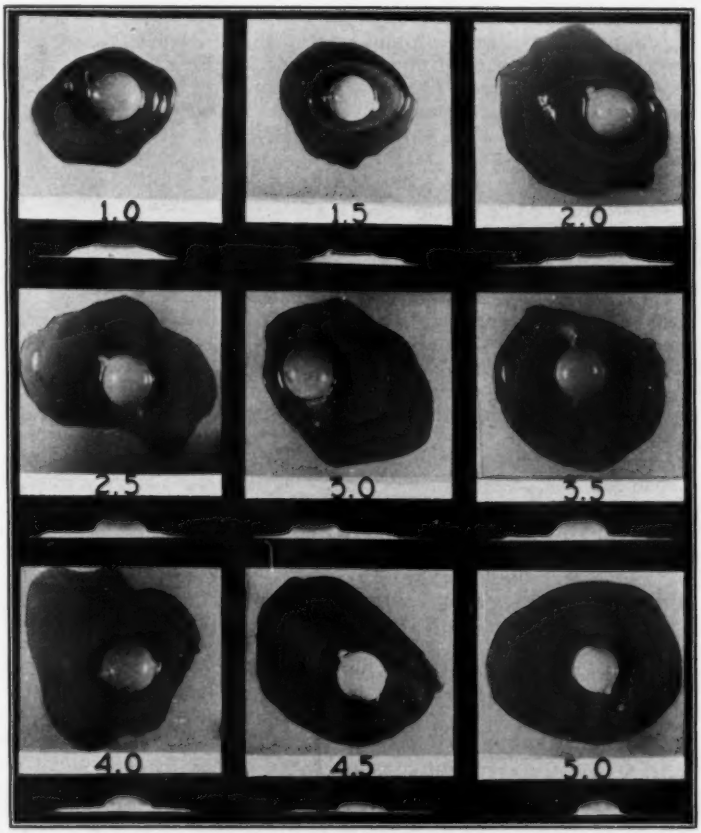

FIGURE 1.—Freshly laid pullet eggs of the Cornell strain of Single Comb White Leghorns scoring 1.0 to 5.0 on the scale suggested by Sharp, showing intervals of 0.5. The eggs shown represent group minima.

In a few instances in which further definition is desirable in exactly placing the score of an egg, the so-called "body" or consistency of the firm albumen may be further determined. This has been done by pouring the firm albumen slowly over the edge of a container and noting its plasticity while retaining the yolk in the container. When quantitative measurements of the albumen are desired, they may be made according to the Sharp (*17*) method of separation by noting the

difficulty with which the firm albumen enters the pipette, or according to the Lorenz and Almquist method (*11*) by noting the apparent plasticity in transferring the egg from the scoring dish to the screen. By observation with candling, using New York State retail grades (*5*), grade Fancy eggs generally score from 1 to 2 when broken out, grade A from 2 to 3, grade B from 3 to 4, and grade C from 4 to 5, but with considerable overlapping.

In the second part of this study, a comparison was made between observed condition of the firm albumen and those factors in candling which would seem to be most closely correlated with albumen condition, in order to determine the relationship between the score for observed condition of the firm albumen and candling quality. A total of 199 eggs was used. These were obtained from several up-State New York City markets and included some fresh eggs from the experiment station flock. Four experienced candlers scored these eggs, and their results were averaged. There was quite regular agreement among the candlers as to the scores for the various factors used. Yolk visibility was scored from 1, for practically invisible yolk shadow, to 4, for a plainly visible yolk shadow. Yolk mobility was scored from 1, for very slight mobility, to 3, for freely mobile yolk shadow. The grades used were from 1 to 8, based upon the New York State retail grades for eggs as described in detail elsewhere (*5*). Eggs were scored as being high or low within each of the New York State grades Fancy, A, B, and C; thus making a complete score of eight grades. No inedible eggs, as detected before the candle, were used. The eggs were opened immediately after candling and were scored for observed condition of the firm albumen and for yolk color as hereinafter described.

In the third part of this investigation, correlations were made between the relative volumes of the three principal layers of albumen obtained as previously briefly described (*19*) and the observed condition of the firm albumen. This was done with data obtained in routine studies in this department on the effect of breeding and feeding upon interior egg quality.

All of the 4,796 eggs studied were from the Cornell strain of Single Comb White Leghorn pullets. All of them were examined on the day that they were laid. Each was carefully broken into a Petri dish, preferably one of 15 cm diameter. If the firm albumen was unruptured, the outer layer of thin albumen was removed into a graduated cylinder by means of a 25-cc pipette. The pipette had a bore of approximately 4 mm, with the tip broken off and the broken end fire-polished. The observed condition of the firm albumen was then noted. Next, this was torn to allow the inner layer of thin albumen to escape. The firm layer was removed from the side opposite the rupture by drawing it into the pipette and was placed in another graduated cylinder. The chalazae and the removable portions of the inner layer of thick albumen, usually very slight, were included in this fraction. Finally, the inner thin layer was removed to a third cylinder. By careful manipulation, accurate and rapid determinations may be made in this manner. The use of moistened cylinders, dishes, and pipettes reduces the error of drainage and speeds up the work.

The yolk was then transferred to a smaller, perfectly flat-bottomed Petri dish for color determination (by the Sharp standards), for yolk-

index measurements (*18*), and, finally, for weight determination. When the firm albumen was found to be in the naturally ruptured state, the observed condition was taken first, and then the firm albumen and finally the combined layers of thin albumen were removed.

EXPERIMENTAL DATA AND DISCUSSION

The results showing the means and correlations obtained between the candlers' score and the opened egg score of the factors studied are given in table 1. The mean observed condition of these eggs was considerably below that of 1.76 found for fresh eggs as shown in table 2. In every case, a significant positive correlation was found between the observed condition of the firm albumen and the yolk shadow visibility, yolk shadow mobility, and candlers' grade. This, therefore, indicates that the condition of the firm albumen is closely related to those factors used in determining the candling quality of the egg. In contrast to this is the report of Almquist (*1*) that there is no correlation between the quantity of firm albumen and the apparent mobility of the yolk, and the report of Pennington et al. (*14*) that there is no relationship between the quantity of firm albumen and the candled grade.

TABLE 1.—*Correlations between candlers' score and opened-egg score of certain factors*

Factors correlated	Mean	$r \pm S.E._r$
Observed condition of firm albumen	2.45	
Correlation of observed condition of firm albumen with—		
Yolk visibility (1=none to 4=plainly)	1.85	+0.504±0.054
Yolk mobility (1=none to 3=freely)	1.77	+ .543± .051
Candlers' grade (1 to 8)	3.66	+ .583± .048
Actual yolk color (00 to 110) sharp standards	58	
Correlation of actual yolk color with yolk visibility (1=none to 4=plainly)	1.85	+ .443± .057

TABLE 2.—*Correlations between the observed condition of firm albumen and the volume of the various layers of albumen*

Condition of firm-albumen sac	Eggs	Factors correlated	Mean	$r \pm S.E._r$
	Number			
Not ruptured	4,531	Observed condition of firm albumen	1.76±0.27	
		Correlation of observed condition of firm albumen with—		
		True firm albumen, percent	53.7	+0.020±0.015
		Outer thin albumen, do	24.4	− .015± .015
		Inner thin albumen, do	21.9	− .006± .015
Ruptured	265	Observed condition of firm albumen	3.38±.47	
		Correlation of observed condition of firm albumen with—		
		True firm albumen, percent	55.2	− .212± .059
		Total thin albumen, do	44.8	+ .178± .059
Do.[1]	261	Observed condition of firm albumen	3.37±.47	
		Correlation of observed condition of firm albumen with—		
		True firm albumen, percent	55.8	− .061± .062
		Total thin albumen, do	44.2	+ .022± .062

[1] 4 abnormal eggs eliminated.

It is interesting to note that the correlation between actual yolk color and visibility of the yolk shadow is significant but not as high

as that between condition of firm albumen and visibility of the yolk shadow. Data obtained with this group of eggs support the opinion of some investigators that the firm albumen is probably of as much or more importance in determining visibility of the yolk shadow than is the actual color of the yolk.

The averages and distribution obtained in the third part of this study for the observed condition of the firm albumen and the proportions of the three chief layers of albumen are shown in table 2 and figures 2 and 3, respectively. The proportions of the various layers of albumen are in general agreement with those obtained by Almquist (*1*). The amount of firm albumen, however, is considerably less than that reported by Card and Sloan (*7*) and by North (*13*). It is interesting to note that the amount of firm albumen was slightly greater in the eggs of poor condition in which the firm albumen sac was ruptured. This apparent increase has also been observed, despite a decided increase in score of condition, in eggs from individuals producing eggs of known interior quality which were stored for periods of from 7 to 10 days.

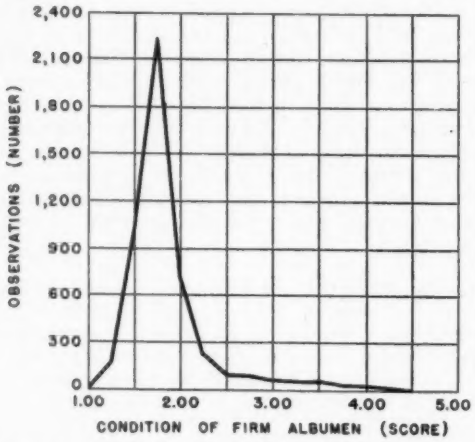

FIGURE 2.—Distribution of the scores for the observed condition of the firm albumen of 4,796 eggs less than 1 day old.

The firm-albumen sac was unruptured in 4,531 of the eggs and permitted the determination of correlations of observed condition of firm albumen with the three main layers of albumen. The results, given in table 2, show that there was not the least correlation between the observed condition of the firm albumen and the proportion of the firm, outer thin, or inner thin layers of albumen.

In the 265 eggs in which this sac was ruptured, the inner thin layer was mixed with the outer thin, hence these eggs could not be included in the larger group. There were slight, though insignificant, correlations between the observed condition of the firm albumen and the proportion of firm albumen and the proportion of total thin albumen, respectively. These slight trends, however, were due to four eggs which possessed an abnormally small amount, or in one case, none, of the firm albumen, and correspondingly large amounts of thin albumen. With these extraordinary observations eliminated, no correlations existed (table 2).

These data present conclusive evidence that the proportion of true firm albumen, used by many investigators as the sole criterion of

albumen quality, is actually in no way related to the firmness, or condition, of the firm albumen. Furthermore, the proportion of inner or outer thin albumen is likewise no indication of the condition of the firm layer.

These findings are in general agreement with the observations of Sharp (*17*) on storage eggs. The two studies cannot be directly compared, however, since he used what he called "apparent thick white", whereas the present study refers to what he defined as "true thick white." The fact that the score of the observed condition of the firm albumen decreases during storage (*8*) and the amount of firm albumen also decreases (*9*) during storage indicates that a correlation should be found between the observed condition and the proportion of the firm albumen of eggs subjected to any considerable period of storage.

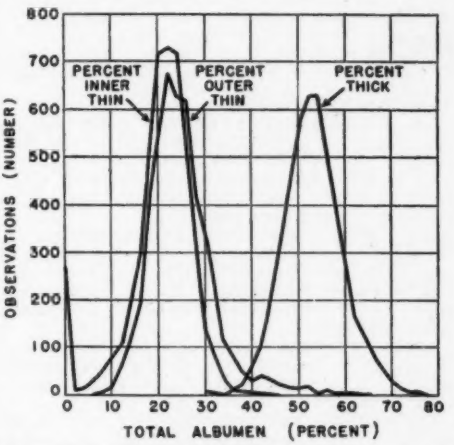

FIGURE 3.—Proportions of the 3 main layers of albumen in 4,796 eggs less than 1 day old.

If the findings of Almquist and Lorenz (*3*), that the firmness of the firm albumen is due to the presence of ovomucin, and those of McNally (*12*), that the firm albumen contains considerably more ovomucin than the thin albumen, are substantiated, the quantitative determination of ovomucin might be used as a more exact measure of the condition of this layer of albumen. It has not been shown, however, that the methods of either authors are quantitative or that the amount of ovomucin is related to the quality or the quantity of firm albumen. That they are not quantitative is indicated by the lack of even moderate agreement in their figures. Furthermore, the use of a chemical method is at a distinct disadvantage when compared with a score such as the observed condition, since the large amount of time involved in a chemical analysis prohibits the use of the number of eggs necessary in studies on egg quality.

This score is not proposed to take the place of more accurate means for measuring this condition when they become available. Some measurements of a physical nature are under study here. One of the most promising of these is the height of the apparent firm albumen weighted for the size of egg and expressed in terms of a 2-ounce egg. The score does, however, offer a valuable aid in studies of egg quality in the interim, and it should supplement such measurements as they become available, since it scores the sum of the factors which constitute albumen quality as seen by the consumer.

SUMMARY

A method for determining the observed condition of the firm albumen of eggs is illustrated. Significant correlations were found between the observed condition of the firm albumen and the visibility and the mobility of the yolk shadow, respectively. Thus, as the yolk shadow appeared more visible and mobile in candling, the score of the condition of the firm albumen was poorer. No correlation was found between the observed condition and the percentage of true firm albumen in freshly laid eggs. It is therefore necessary to measure the condition as well as the quantity of firm albumen in the egg.

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THE DETERMINATION OF SPRAY COVERAGE ON APPLES[1]

By KERMIT GROVES, *assistant chemist*, and JAMES MARSHALL, *assistant entomologist, Washington Agricultural Experiment Station*

The importance of determining the deposit of insecticides on fruit in spray investigations seems to have been underestimated by many workers in this field. Published reports have frequently given comparisons of the effectiveness of spray programs without stating the quantitative and qualitative nature of the deposit of the insecticide. It often happens that an effective insecticide gives poor control simply because very little of it remains on the fruit.

The physical and chemical properties of powdered insecticides differ greatly. The density of calcium arsenate is about 2½ g per cubic centimeter, while that of lead arsenate is nearly 6 g. It cannot be assumed that different insecticides, mixed in the spray tank in the same way, will be deposited in the same quantities on the fruit surface. Yet the weight of the material per 100 gallons of water is frequently the only basis for comparison employed by investigators. Comparisons of these weights give useful economic information, but they should not be relied upon for comparisons of control. Repeated analyses of representative samples of the sprayed fruit to determine the mass of insecticide per unit area seems to be the logical basis of comparison. This paper describes the methods used at the Washington Experiment Station for the determination of arsenical deposit on apples.

Frequently more than a third of the insecticide deposit on an apple is on the depressions of the calyx and stem ends. If the total deposit is determined and the coverage is expressed as the average deposit per unit area, the result has little meaning. It is much higher than the deposit per unit area on the cheek surface and lower than the deposit in the ends. In table 1 are shown the results of coverage determinations of arsenical sprayed apples. There were 12 apples in each sample, and the cheek surfaces and the ends were analyzed separately. The arsenic in the stem and calyx ends varies from 24 to 42 percent of the total arsenic on the apples. A variation of this percentage renders the average deposit determined from the total deposit unreliable. The ends should be eliminated or analyzed separately.

The estimation of the area of the apples is the chief difficulty in determining coverage. Apples may be considered as spheres and their areas calculated from average diameters. This approximation may be sufficiently accurate for comparisons when the apples are similar in size and shape. A better approximation for certain varieties of apples, such as Winesap and Jonathan, can be made by calculating the area of the surface of revolution of a cardioid about its axis of

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symmetry. Barnes [2] computed the area of apples by the use of this geometric figure, using the equation

$$\text{Area in square inches} = 0.755 \left(\frac{1}{D}\right)^{2/3} W^{2/3}$$

where W is the weight of the apple in grams and D its density.

Perhaps a better method is as follows: The area of the surface of the revolution of the cardioid,

$$\rho = a(1 - \cos \theta)$$

is approximately 20.1 a^2. a is determined from the apple measurements by multiplying the diameter perpendicular to the core by 0.385, and the diameter parallel to the core by 0.444 (fig. 1). Several Winesaps, picked in midsummer, were measured in this manner and their areas calculated; the peel was then removed in thin panels and the areas measured with a planimeter. The calculated and measured areas agreed within 3 percent. The stem and calyx ends may be removed with a cork borer and the areas removed deducted from the total area. The arsenic deposit may be removed from the apples with an alkaline-soap solution and acid rinse,[3] the solution boiled down and digested with sulphuric and nitric acids,[4] and the arsenic determined by the bromate method.[5]

TABLE 1.—*Distribution of deposit on apples*

Sample No.	Arsenic (As_2O_3)				Sample No.	Arsenic (As_2O_3)			
	Stem and calyx	Cheeks	Total	In ends		Stem and calyx	Cheeks	Total	In ends
	Milligrams	*Milligrams*	*Milligrams*	*Percent*		*Milligrams*	*Milligrams*	*Milligrams*	*Percent*
1	4.04	8.42	12.46	32.4	22	8.36	11.46	19.82	42.2
2	3.69	5.96	9.65	38.2	23	5.41	11.33	16.74	32.3
3	3.32	4.90	8.22	40.4	24	5.67	11.00	16.67	34.0
4	3.60	7.16	10.76	33.5	25	7.55	18.24	25.79	29.3
5	3.74	8.74	12.48	30.0	26	4.33	8.52	12.85	33.7
6	5.30	12.16	7.46	30.4	27	7.42	13.04	20.46	36.3
7	5.43	9.35	14.78	36.7	28	4.83	7.80	12.63	38.2
8	3.91	8.04	11.95	32.7	29	5.89	9.40	15.29	38.5
9	6.73	14.25	20.98	32.1	30	5.64	10.00	15.64	36.1
10	4.34	10.17	14.51	29.9	31	7.13	12.66	19.79	37.0
11	3.48	8.11	11.59	30.0	32	7.92	14.70	22.62	35.0
12	8.18	15.88	24.06	34.0	33	4.54	6.63	11.17	40.6
13	5.49	14.34	19.83	27.7	34	5.04	7.03	12.07	41.8
14	5.54	9.86	15.40	36.0	35	6.81	10.13	16.94	40.2
15	3.92	8.71	12.63	31.0	36	6.72	10.52	17.24	39.0
16	2.83	7.51	10.34	27.4	37	4.20	11.55	15.75	26.7
17	3.75	7.12	10.87	34.5	38	3.31	7.37	10.68	31.0
18	3.45	7.41	10.86	31.8	39	3.04	7.74	10.78	28.2
19	3.42	7.77	11.19	30.6	40	3.89	7.13	11.02	35.3
20	3.73	8.71	12.44	30.0	41	4.57	7.82	12.39	36.9
21	3.11	7.25	10.36	30.0	42	1.83	5.83	7.66	23.9

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A simpler method of determining coverage consists in cutting a number of disks from the fruit with a cork borer and analyzing them. The area of a disk removed from a sphere may be calculated by the formula,

$$\text{Area of disk} = 2\pi R\ (R - \sqrt{R^2 - r^2})$$

where R is the radius of the apple and r is the radius of the disk. It is not necessary to calculate this area for each different apple radius; a table of areas may be calculated for each quarter- or half-centimeter radius interval and the areas of the disks taken from the nearest calculated radius. The disks are then digested [4] and the arsenic

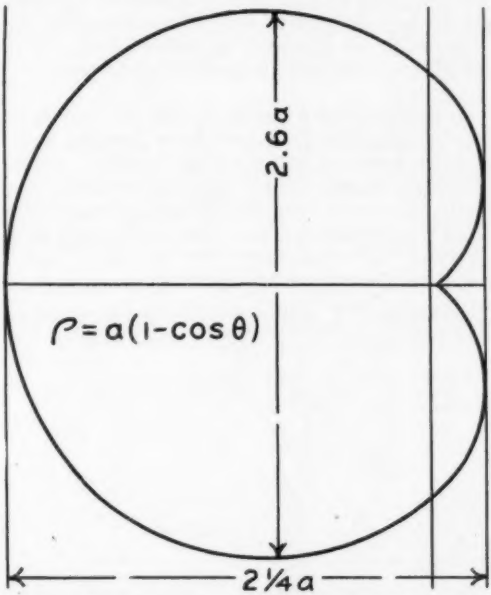

FIGURE 1.—Diagram showing the dimensions of a cardioid in terms of a.

determined by the bromate method.[5] The digestion requires very little time.

In the work at this station samples of 12 apples were selected from each experimental plot and 6 or more disks were cut from each apple for analysis. A sample of this size proved satisfactorily reproducible. Barnes [6] recommended samples of 50 apples each in order to obtain a result with a probable error of 5 percent. However, Barnes was interested in the deposit from a residue standpoint and the calyx and stem ends were analyzed with the cheek surfaces. The elimination of these ends, which is essential for accurate coverage information, reduces the variation considerably. It may be well to add that this source of variation is but one of several which occur between the application of spray and the eventual determination of coverage.

[4] See footnote on page 1143. [5] See footnote on page 1140. [6] BARNES, J. W. See footnote 2.

For example, the type of spray mixture is of the utmost importance in effecting uniformity of spray application. Certain arsenical spray mixtures yield deposits which quickly reach a maximum as spraying is prolonged, whereas others recently developed at this station have the capacity of leaving deposits that increase almost indefinitely with prolonged spraying. Thus the careful and systematic application of spray materials so necessary in any case becomes doubly important. Should one tree be sprayed, let us say, twice as heavily as its neighbor the amounts of the arsenical spray deposits on the two trees might readily vary 100 percent from this source alone.

Apples selected for analysis must be handled carefully and the surfaces to be analyzed should not be touched. The apples should be held by the stem and calyx ends. While being transported to the laboratory they may be impaled on nails driven through boards. A cork borer with an inside diameter of about 1.8 cm is convenient for disk cutting.

According to the writers' experience, the Gutzeit method, though useful for determining minute amounts of arsenic, such as occur in residue samples, is less suitable than the bromate method for ascertaining the relatively large amounts which are encountered in measuring deposits. In the latter case the 10 percent error expected in the Gutzeit method [7] compares to less than 1 percent in the bromate method.[8] Coverage determinations by the disk method correlated unusually well with control on arsenical sprayed plots.

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